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POTATO HAULM DESTRUCTION WITH SPECIAL REFERENCE TO THE USE OF TAR ACID COMPOUNDS

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(With 1 Text-figure)

The chief aim of the investigation was to find an effective non-corrosive spray for killing potato haulm.

Certain tar acids were nearly as effective as sulphuric acid, but as some samples of such compounds were highly irritant, only those proving both effective and relatively harmless to the human skin were passed as suitable.

The chemical composition of a sample of tar acids is largely dependent on the method of carbonization employed. The samples examined were from horizontal-retort, coke-oven, vertical-retort and low-temperature processes. At present, suitable compounds which can be produced commercially have been obtained from the two last-named only. Fractionation at intervals of 10° C. indicated that, as a rule, high-boiling fractions (250–290° C.) were more effective haulm-killing agents and less irritant to the skin than low-boiling fractions (190–250° C.).

Various methods of emulsification are discussed. An emulsifier, consisting of a mixture of gum arabic and casein, was developed, yielding a stable concentrate, easy to dilute with water of any degree of hardness. The spray when applied formed a tenacious rain-resistant film on the leaves. The action of sulphuric acid was considerably reduced by rain falling shortly after application.

Sodium chlorate gave a fair leaf kill but a poor stem kill and is, therefore, far less effective than sulphuric acid. Restricted trials carried out with calcium cyanamide dust indicated that this compound is even less efficient than sodium chlorate.

Di-nitro-cresol derivatives were only moderately effective and have objectionable staining properties.

Of the copper compounds tested, copper chloride and nitrate proved the most effective but were not as good as sulphuric acid and are relatively expensive. A mixture of copper sulphate and salt was moderately effective on senescent haulm only. Like sodium chlorate it has the advantage of being inexpensive.

Tar-oil winter wash proved effective, but is expensive and slow in action.

Young haulm is readily killed but becomes more resistant as the season advances and finally again susceptible during senescence. Individual leaves on a plant show the same age/susceptibility relation. Varietal differences in susceptibility appeared to be largely the result of varietal differences in maturity.

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Control of blight in tubers was related to the extent of haulm kill obtained by spraying. Unless the fungus was actually wetted by the spray, it continued to sporulate freely on the leaves until the tissues died. For this reason slow-acting compounds, such as tar-oil winter wash and those which are ineffective on stems, provide less adequate control than quick-acting, highly effective compounds such as sulphuric acid and tar acids.

'Setting' of the skin of tubers is accelerated by killing the haulm, but the kill must be virtually complete. Therefore, to obtain maximum resistance to lifting injury, the crop should be left in the ground as long as possible after spraying. Methods of assessing 'skin setting' are described.

Cooking tests on the produce of plots where the haulm had been killed by various tar derivative and other compounds showed no deleterious effect on quality. Neither was there any indication of detrimental residual effects in the soil following spraying with various tar-acid and copper compounds, tar-oil winter wash, sodium chlorate (3%) or sulphuric acid.

A low-temperature tar-acid compound, T.A.C. 36*c*, incorporating a gum emulsifier, gave satisfactory results in large-scale tests made by War Agricultural Executive Committees in 1945. This compound, with a slightly improved emulsifier (gum arabic + casein), reference T.A.C. 46, and a vertical retort tar-acid compound, T.A.C. 42, proved equally successful in a restricted field trial and are regarded as satisfactory for commercial use. Because of present cost of materials (40*s*. per acre for a 100 gal./acre application of 10% spray) their use is likely to be restricted. Comparative costs of the more effective compounds are given.

The most effective non-corrosive substitutes for sulphuric acid on vigorous haulm are certain tar-acid sprays. For killing haulm at the end of the season some of the less effective and cheaper compounds, such as sodium chlorate or copper sulphate and salt, may prove reasonably satisfactory in most years.

INTRODUCTION

The destruction of potato haulm just prior to harvesting the crop is now carried out annually on many thousands of acres. It helps to prevent the infection of tubers at lifting time with blight, caused by the fungus *Phytophthora infestans* (Mont.) de Bary, facilitates lifting by removal of haulm which would clog the machines, and destroys weeds. In seed-growing areas, haulm destruction may also be carried out earlier in the season, to increase the proportion of seed-sized tubers and to prevent late virus infection after the crop has been inspected.

Haulm killing as a blight control measure is a comparatively recent introduction, although Large (1940) mentions that cutting of the haulm was first advocated by Morren in Belgium within a fortnight of the first disastrous outbreak of blight in 1845. The first recorded attempt to destroy potato haulm by chemical means was carried out in 1931 in Holland by Van Poeteren (1932), who used 5% copper sulphate and 1% sodex or agricultural salt. In 1932, many experiments on weed control by sulphuric acid were made in England as a result of successful trials in France, and in that year Mr E. H. I. Brown and Dr H. C. Brown of the West Norfolk Farmers Manure and Chemical Co-operative Co., Ltd., King's Lynn, working in a district where it was a common practice to remove potato haulm by means of a grass cutter, conceived the idea of using sulphuric acid for the complete destruction of the haulm. Continuing their experiments in 1933, they obtained successful results by the application of the acid mainly at a strength of 15%, but their conclusions were never published. Following up this work, trials were undertaken in

1933 and 1934 by the National Sulphuric Acid Association Ltd. (Anon. March 1935), in which the acid was used at 10 and 20%. The results of a randomized block experiment in 1934 showed that a considerable reduction in tuber blight had been effected. Figures obtained in this test are quoted by MacDowall (July 1935). In 1934 also, Bates & Martin (June 1935) obtained similar satisfactory results in a critical field trial with sulphuric acid, and indicated that the costs involved were amply justified even when the blight attack was only slight. Small (1935*a*), who had reported on the effectiveness of a copper sulphate-caustic soda mixture in Jersey, later (Aug. 1935*b*, 1936) found that the action of sulphuric acid was more satisfactory.

As a result of these trials, the use of sulphuric acid for haulm killing became more widespread, and even in 1934 some 3500 acres of potatoes in Norfolk, Lincolnshire and Scotland were sprayed and an excellent control of blight claimed. Neuweiler (1940) noted from Switzerland that although sulphuric acid is an efficient haulm-killing agent it may cause a slight reduction in yield. The extent of any such reduction would obviously depend upon the stage of growth of the haulm when destroyed.

Attempts to find an alternative to sulphuric acid were made by Findlay & Sykes (1936), who stated that copper sulphate applied both as a spray and a dust was as effective as the acid. This was not borne out by their further work (1937) nor by unpublished results obtained by other investigators. In the years immediately prior to 1939, sodium chlorate and calcium cyanamide were tried, apparently with promising results, but both these chemicals became unobtainable under wartime conditions. Several proprietary compounds, some containing copper salts and others di-nitro-cresol derivatives, which had been used with satisfactory results as weed killers, were also claimed to be effective haulm-killing agents. Tar-oil winter wash was also used with some success, but its action on the haulm was slow. Tests were carried out with all these compounds during the present investigation, and the results are given later.

Several American workers, including Schultz *et al.* (1944) and Fernow & Smith (1944), have claimed successful results from applications of sodium di-nitro-ortho-cresylate + ammonium sulphate. The latter workers also obtained an effective kill using a 6% solution of sodium pentachlorphenate, which is an irritant and may blister the skin, and calcium cyanamide and sodium di-nitro-ortho-cresylate dusts. Amongst the other compounds which they tested were sulphuric acid, ammonium sulphamate, certain chlorates and arsenites and the sulphates of copper and iron.

There is no doubt that sulphuric acid or brown oil of vitriol, applied at a suitable strength, is the most effective compound known at present for the destruction of potato haulm, and a large acreage is burnt off annually with it in this country.

There are, however, several objections to the use of sulphuric acid, such as the discomfort caused to those engaged in the spraying operation, the necessity for protective clothing and acid-resistant spraying machinery, and the general difficulty of handling a highly corrosive liquid. These disadvantages have been the main

factors in preventing the wider adoption of haulm killing, particularly among smaller growers. Spraying with sulphuric acid is carried out mainly on contract, but since it is doubtful whether the acreage on which haulm killing would be a desirable practice could be covered by contractors alone, the advent of a cheap non-corrosive compound approaching sulphuric acid in effectiveness, especially of stem kill, but without its inherent disadvantages, would be of great value and might result in a considerable increase in the acreage of potato haulm destroyed annually.

The present investigation was begun during the war, when sodium chlorate and calcium cyanamide were unobtainable and when a substitute non-corrosive chemical for haulm killing would have been most valuable. It has been shown that certain tar acids can be compounded into preparations approaching sulphuric acid in speed of action and effectiveness. It remains to be seen whether these will find favour commercially in competition with the less effective, but cheaper, chlorate, which is now again on the market.

METHODS

Field tests

Initially, tests were carried out on $\frac{1}{400}$-acre plots, but as the number of compounds increased it became necessary to carry out routine trials on a smaller scale, and plots consisting of six plants, three in each of two adjacent rows, were found adequate for the purpose. Where finer differences in effect were sought, randomized block schemes of six-plant plots and Latin square arrangements of $\frac{1}{400}$-acre plots were employed. On plots of up to $\frac{1}{400}$ acre the compounds were applied by means of a hand-pressure sprayer which was found very efficient for the purpose, while on larger plots the knapsack type of sprayer was used. The most successful compounds were applied to larger areas, up to $\frac{1}{2}$ acre, by the normal tractor or horse-drawn commercial spraying machine.

In the autumn of 1944, two tar-acid compounds (T.A.C. 2*a* and 4) which had been found very effective in plot trials, were tested under commercial conditions in conjunction with War Agricultural Executive Committees in many parts of the country. Notes on the results have been published elsewhere (Anon. 1945). In 1945 similar trials were carried out with an improved compound (T.A.C. 36*c*), and the results of these are discussed later (p. 16).

Application of the sprays was usually at the rate of 100 gal./acre, but in some tests, where the haulm was very tall and vigorous, this rate was obviously inadequate to give a complete cover, and consequently the application was increased to 150 or even 200 gal./acre. Most of the inorganic and some of the organic compounds were tested initially at a concentration of 5%, while most of the tar acids were used at 5–15% of the stock emulsion as well as at 5% of their tar-acid content.

Each set of tests included untreated control plots, so that the natural decline in vigour of the plants with maturity could be observed. The necessity for this will be

discussed later. Plots sprayed with 10% sulphuric acid were also included as standards, while plots sprayed with 7% tar-oil winter wash and 3% copper sulphate + 1% salt were introduced on occasion to determine degrees of efficiency.

The chief varieties of potato used were Majestic, Doon Star and King Edward, but some tests were carried out with the late-maturing variety Dunbar Standard, which is noted for its tall and very vigorous haulm. In one experiment one of the tar-acid compounds was applied to plots of fifteen different commercial varieties.

Many compounds are very effective in destroying the leaves while the stems remain practically undamaged, thus complicating comparisons of effectiveness. The method of recording adopted in the field tests, therefore, was an eye estimation of leaf and stem kill separately, on a percentage basis in intervals of 5%. The additional ratings of 2 and 98% were, however, included, since a finer eye estimation is possible near the upper and lower limits. This method was found to be both rapid and capable of giving reproducible results, as shown by the comparison of independent observations by the same and different workers. These observations rarely differed by more than 10%. In this connexion it is interesting to note that Townsend & Heuberger (1943), working on celery early blight (*Cercospora apii*), found that the visual method of assessing losses in plots due to the disease was just as accurate and infinitely more rapid than the method of classifying all leaves from individual plants into categories according to the amount of disease present. Experience in the haulm-killing experiments has shown, however, that estimates of effectiveness of any given compound tend to be lower in small plots than in large-scale field trials.

For the purpose of comparing the effect of compounds which had been tested at different times during the season (Appendix 3), individual percentage kills were calculated as percentages of the kill given by 10% sulphuric acid applied in the same test (see p. 16).

Laboratory tests

In view of the large number of substances involved in the trials, the relatively short season available for field tests and the time, labour and acreage of potatoes required, various attempts were made to develop a satisfactory laboratory test of haulm-killing efficiency. The intention was not to supersede the field-plot tests but to use a laboratory method for a preliminary sorting out of tar-acid compounds submitted, thus enabling more comprehensive field tests of those showing promise to be undertaken than the time and labour factors had previously permitted.

Tests were made with (*a*) leaf disks of 1 cm. diameter, (*b*) leaflets and (*c*) whole leaves. In each case the method of application was by dipping the plant material in a container of spray fluid of the desired strength. Evaluation of effect was either (1) by length of time taken to obtain 90% discoloration of the tissue after dipping in a standard concentration of tar acids, or (2) by determining the maximum dilution of tar acids causing 90% discoloration after a given period, normally 2 hr. In each case it was found preferable to expose the treated plant tissue to the air

rather than to enclose it in a Petri dish or other container. The leaf disks were placed on filter paper after dipping.

Tests (*a*2) and (*b*2) were found the most promising combinations, and work was, therefore, concentrated on these two methods. The concentrations of tar acids normally used were 5, 2·5, 1·25 and 0·625%. Altogether some 200 tests were carried out involving approximately sixty compounds.

From the outset it became plain that the main difficulty was the considerable variation in susceptibility between individual plants and between leaves of different ages on the same plant, superimposed upon which was a seasonal variation. Consequently, despite all possible precautions, such as the use of leaves of approximately the same age, results were not reproducible with any degree of accuracy. The question of susceptibility is dealt with elsewhere in this paper. Compounds which proved highly efficient in the laboratory tests invariably proved efficient in the field, but the converse, unfortunately, did not apply. From the beginning it was realized that these tests could give no indication of the efficiency of slow-acting compounds such as tar-oil winter wash, sodium chlorate, copper compounds, etc., but this point seemed immaterial when working with tar-acid compounds, where efficiency and speed of action appeared to be closely related. Subsequently, however, results indicated that the tests were discriminating against compounds which were efficient and only slightly slower in action than normal. As the length of time that it is possible to retain cut plant tissue in a suitable state is limited, no solution of the difficulty by taking readings after a much longer interval appeared possible.

In view of the difficulties enumerated above it was decided that considerably more work would have to be done before a reliable laboratory test could be evolved; therefore, the field-plot tests, despite their disadvantages, were retained as the only available criterion of efficiency of haulm-killing compounds.

EXPERIMENTAL

I. *Compounds other than tar acids*

In the initial experiments many well-known corrosive compounds and mixtures of substances both organic and inorganic were used. A complete list of those which were without effect, whose action on potato haulm was unsatisfactory or which were discarded for other reasons is given in Appendix 1. Notable amongst these are solutions of acetic acid, hydrochloric acid, phenol, calcium hypochlorite and ferrous sulphate, all of which were virtually without effect. Sodium hypochlorite solution had a moderate action on the leaves but left the stems practically untouched. Ammonium thiocyanate, in a 5% solution, gave a very efficient leaf and stem kill, but its use in practice is undesirable by reason of its toxicity. Sodium hydroxide, although slightly less active, was also relatively efficient but must be dismissed on account of its corrosive nature.

The application in the form of dusts, to both wet and dry leaves, of certain

compounds which were relatively efficient in solution gave unsatisfactory results, and work upon this line was discontinued.

The suggestion has been made that a flame gun of the type employed for weed killing, or some modification of it, might be used for haulm destruction. Tests showed that such apparatus would be totally inadequate with applications even considerably longer than would be economically possible.

Sulphuric acid

Sulphuric acid has proved the most effective compound tested, taking the average of the results obtained throughout the trials. For this reason, as previously stated, it has been used as a standard for comparison. The rate of application in commercial practice varies with the size of the haulm, and the season from 10 to 20 gal. of brown oil of vitriol (sp.gr. 1·7 or 77% sulphuric acid) in 80–120 gal. of the spray per acre. Recently devised machinery permits of the application of concentrated acid at the rate of approximately 20 gal./acre, thus obviating cartage of water. This method is at present confined to contractors on account of both the danger of handling concentrated acid and the expense of the special equipment.

Sulphuric acid causes a rapid wilting of the leaves; at the same time the petioles frequently develop a marked reddish tinge. The leaflets themselves and then the stems become light brown and finally bleach in a characteristic manner which renders haulm sprayed with this compound easily distinguishable. The leaves and tops of the stems are bleached and dry out in a period of from 3 to 7 days, but with the strengths of acid commonly used, green areas are usually present lower down on the stems which sometimes remain in this condition for as long as 21 days.

It is sometimes stated that plants sprayed with 10–20% sulphuric acid are killed within 24 hr. This has not been experienced during the three years of experimental work described in this paper. Even where plants were relatively senescent at the end of the season they were rarely completely killed in less than 10 days.

Sodium chlorate

Sodium chlorate has been used in solution at rates up to 30 lb./100 gal./acre, but beyond a rate of 15 lb./100 gal./acre the improvement in kill was relatively slight. Speed of action was only moderate, little effect being visible in less than 24 hr. Despite a fair leaf kill, this compound cannot be regarded as fully satisfactory on account of its poor action on stems. For this reason also it is not comparable with sulphuric acid and many of the tar-derivative compounds tested.

Calcium cyanamide

Calcium cyanamide was found an efficient haulm-killing agent by Fernow & Smith (1944) in the United States, when applied in the form of a dust at the rate of 40–60 lb./acre. In limited trials carried out in the course of the present investigation, it has been applied as a dust at rates up to 2 cwt./acre. The results obtained

indicate that this compound, while similar in action to sodium chlorate, is considerably less effective. Applications to previously wetted leaves gave slightly better results than to dry leaves presumably due to better retention of the dust.

Di-nitro-cresol derivatives

Successful haulm destruction with certain di-nitro-cresol derivatives, normally used for charlock eradication, has been claimed by some investigators. Sodium di-nitro-ortho-cresylate mixed with ammonium sulphate as an 'activator', stated to be effective by Schultz *et al.* (1944) and Fernow & Smith (1944), working in the United States, has been tested during the present investigation at the rate of 10 lb. of the cresylate + 10 lb. ammonium sulphate, in 100 gal./acre, but even when this strength of spray was doubled, haulm kill was only moderate and was invariably most uneven. Similar results have been observed in War Agricultural Executive Committee trials with di-nitro-ortho-cresol. Di-nitro-cresol derivatives have the serious disadvantage of being persistent yellow dyes, and since, by reason of their inflammability, they are normally marketed in the form of a paste, making up the sprays in the field is a lengthy and awkward process.

Copper compounds

Copper sulphate alone, even when used as a 5% solution, was found to have little effect on vigorous potato haulm, although a spotting or burning effect was often noted on senescent haulm. The addition of 1% common salt to a 3% solution of the sulphate, however, resulted in a marked increase in efficiency, especially when plants were approaching maturity. On green, vigorous haulm, even this mixture, which was found to be the optimum consistent with economy, had virtually no effect whatsoever. At no time was copper sulphate and salt found to give a satisfactory stem kill. No apparent benefit was obtained by the incorporation of a spreader (sulphonated lorol).

Cupric chloride and copper nitrate, used at 3%, were both moderately efficient, apparently more so than the 3 : 1 mixture of the sulphate and salt. Again no improvement was obtained by the addition of a spreader. By increasing the strength of both the cupric chloride and copper nitrate sprays to 5% results were improved, but it is most doubtful whether this would be an economic dosage for large-scale use, even if further trial gave results warranting such a course.

The mixture of 3% copper sulphate and 2·5% sulphuric acid has been used, apparently to good effect, in Northern Ireland, but in the present tests, although proving fairly satisfactory, it appeared to have no advantage over sulphuric acid alone. The burning and bleaching action of the acid on the haulm was a noticeable effect of treatment with this mixture.

Copper compounds cause an initial marginal curling of the leaflets, followed by a general shrivelling, the foliage becoming greyish brown. The stems become spotted and discoloured but remain turgid for a considerable time, even where

a complete kill is eventually obtained. Neither in speed of action nor in effect can these compounds be compared with sulphuric acid and certain of the tar-acid compounds discussed below.

Tar-oil winter wash

Tar-oil winter wash having a phenol content not exceeding 10% is produced commercially for spraying fruit trees. In recent years it has been tried on a field scale for potato-haulm killing but has been found very slow in action. Confirmation of this was obtained in the present work. The usual rate of application was 7 gal. of the stock emulsion in 100 gal./acre. The immediate effect on the plant was that the midribs and lateral veins of the leaves became dark brown and the upper surface of the leaflets assumed a shiny, dark or bronzed appearance. The under-surface remained green and no wilting took place for about 7 days, after which fairly rapid shrivelling occurred. The stems gradually turned brown but remained turgid for a considerable time. So long as the under-surface of the leaflets remained green it was observed that the blight fungus could remain there in an actively sporing condition. Consequently the slowness of action of this material, combined with its rather high cost, militates against its effectiveness and success as a haulm-killing agent.

II. *Tar-acid compounds*

The slow but satisfactory results given by tar-oil winter wash in early trials of substitutes for sulphuric acid suggested that a systematic examination of tar oils might reveal some quicker acting and more effective product. In preliminary experiments carried out in 1943, tests were carried out with two high-boiling tar-acid compounds, subsequently designated T.A.C. 1 and 2, and three creosotes, light, medium and heavy. Although none of the creosotes could be regarded as satisfactory the gradation in effectiveness was very noticeable, the greatest effect being produced by the heavy type. Both of the tar acids on the other hand provided a remarkably rapid and complete kill of haulm, T.A.C. 2 being, in all tests, slightly more effective than T.A.C. 1. These tar acids were made up in sodium oleate emulsion and were applied at 5% of the tar-acid content.

The success obtained with these tar-acid compounds, which have the advantages of availability and non-corrosiveness to metal, led to the concentration of further work upon this group of substances.

Origin

The products tested in these experiments (Appendix 2) included materials derived from the oils produced by the distillation of the tars from low-temperature, vertical retort, coke-oven and horizontal retort carbonizations. Some constituents of these oils are soluble in caustic soda, and on treatment of this alkaline solution with acid, 'tar acids' separate out. 'Tar bases' remain and may be isolated by

treatment of the oil with acid and subsequent neutralization of the solution. The oil insoluble in acid or alkali is referred to as 'neutral oil'.

The tar acids were by far the most active materials for haulm destruction. They were obtained direct from the distillers, and in many cases exhibited a wide boiling range. Advantage was taken of this to prepare, from representative samples obtained by different types of carbonization, fractions at 10° C. intervals. These were used in field tests in an attempt to determine the range of maximum potency. It must be emphasized, however, that since no elaborate fractionation was attempted, the temperature range can only be regarded as a rough guide. Tar acids consist of a range of phenolic substances of different boiling-points. Differences in chemical constitution may exist between many of these compounds having equal boiling-points but obtained from different sources, e.g. from the low-temperature and horizontal-retort processes. Even those obtained from the same source may vary in chemical nature. Striking instances of the difficulties arising from this will be given later.

In a few cases tar oils (T.O.C. 11, 14, 17, 20) and tar bases (T.B.C. 23, 24) were tested, but compared unfavourably with the more efficient tar acids. T.A.C. 3, 5, 7, 8, 9 and 10 were commercially available products of various types which also proved ineffective (Appendix 3).

Emulsification

In all but three cases (T.A.C. 8 and 9 and T.B.C. 23) the products to be applied to the plants were oils insoluble in water, and in order to obtain the desired dilution for application it was necessary to prepare them as emulsions. Two types of concentrated emulsion were used, namely, 'miscible oil' and 'mayonnaise'; these concentrates were diluted with water to the required strength before use.

Miscible oil. This term is applied to clear liquids which yield emulsions on dilution with water. They are usually systems of four components, the oil, the emulsifier, water and a constituent which has been variously called a 'co-solvent', 'aid to dissolution' or 'peptizer'. The usual peptizers are fusel oil, alcohols, cresylic acid and particularly meta-cresol. The tar acids themselves have a considerable peptizing action, and in the preparation of miscible oils of these materials a separate peptizer in the system is not required. Thus, where sodium oleate is used as emulsifier, the miscible oil is best prepared by dissolving the oleic acid in the tar acids and then adding rather less than the equivalent of caustic soda required, as a 40% solution (w/w) in water, to form sodium oleate. The clear liquid obtained is then tested to determine stability on storage, degree of dispersion on dilution with water and the stability of the dilute emulsion. Ease of preparation of the dilute emulsion is definitely increased by having a slight excess of the fatty acid present. Different materials require varying amounts of emulsifier, and exact conditions can only be determined by experiment. Examples of this type are given in Appendix 2 (T.A.C. 28–32).

Mayonnaise. This is made by preparing the oil phase and water phase separately and then mixing by gradual addition of the oil to the water during agitation. The emulsifier is more generally in the water but may be in the oil phase. In the small-scale preparations this preliminary mixing can be carried out by hand stirring and the mixture then passed through a cream machine. On a large scale the oil phase is added slowly to the aqueous phase which is circulated in a jet-type homogenizer (B.P. 407153/1934 by Gas Light and Coke Co., Murdoch and Shannon). This machine has the special advantages that it causes little or no foaming, and circulation of the mixture can be continued until the required degree of dispersion has been achieved; at all times the water phase is in excess.

Sodium oleate itself produces stable emulsions giving good dispersion of tar acids, particularly if the oleate is formed *in situ*, e.g. if oleic acid is dissolved in the tar acids and caustic soda added to the mixture. Emulsions using sodium oleate alone, however, suffer from the disadvantage that if hard water is used for dilution, soluble soap is removed from the system, and unless a sufficient excess of oleate has been used in the first place the emulsion will at once break. Many examples of emulsions containing sodium oleate alone are listed in Appendix 2. These were satisfactory for small-scale work when distilled water was available for dilution, but they cannot be regarded as suitable in field use.

Later combinations of sodium oleate and a stabilizer, such as S.R., gave emulsions more stable to hard water, but these materials, which were mainly miscible oils (T.A.C. 33*a*, 34*a*, 35*a*, 35*d* and 36*a*) required to be mixed with an equal volume of water before dilution, and this would be impracticable in the field.

A combination of Perminal E.M.L. and casein, which was used in field trials in 1944 (T.A.C. 2*a* and 4), was a more satisfactory material, but even with this, care was necessary in the preliminary mixing. It was obvious that a concentrate was required that could be poured direct into the requisite amount of water, and consequently further work was done to develop an emulsion of this character.

During these investigations a proprietary vegetable glue was included as emulsifier, and a mayonnaise containing 3% of glue, 60% of tar acids and 37% of water gave an excellent concentrate which could be added to water or vice versa, requiring only a quick stir to ensure a satisfactory dispersion. Glassware in which the gum emulsions were prepared retained an opaque film even after prolonged washing under a cold tap. This observation suggested that a toxic film of tar acids could be placed on leaves which would resist the leaching action of rain. Experiments to be described later confirmed this view (Tables 5, 6). This emulsifier was used in large-scale field trials in 1945 (T.A.C. 36*c*).

Further work showed that gum arabic (1·5%) emulsions displayed the same properties as the glue. With both the gum arabic and proprietary glue emulsions a water layer tended to separate out when the concentrate was stored for several days. Although redispersion was easily effected by gentle shaking, there was a possibility that concentrates might have to be stored for 12 months or more and

redispersion might then be difficult; consequently some improvement in the stability of the gum arabic emulsion was necessary. Sodium cascinate emulsion had also shown a water-resistant film, but the concentrate, although stable, could not be diluted easily. However, on combining gum arabic and sodium cascinate a concentrate was obtained which retained the desirable qualities of both. The most satisfactory formula which was used in the preparation of the compounds finally recommended (T.A.C. 42 and 46) is given in Table 1. A provisional specification of the application of this method of emulsification to haulm-killing sprays and tar-oil winter wash has been filed with the Patent Office (27902/45).

TABLE 1. *Typical formulae of emulsion concentrates (percentage by wt.)*

Type	Tar acids	Oleic acid	Sodium hydroxide 40%	S.R.	Proprietary gum	Gum arabic	Cascin	Water
(*a*)	80·0	12·96	3·2	3·84	—	—	—	—
(*b*)	50·0	—	—	5·0	—	—	—	45·0
(*c*)	60·0	—	—	—	3·0	—	—	37·0
(*h*)	60·0	—	0·05	—	—	1·0	0·5	38·45

Type (*a*) = typical 'miscible oil' emulsion.
Type (*b*) = typical 'mayonnaise' emulsion.
Type (*c*) = typical formula using proprietary gum.
Type (*h*) = formula using mixture of gum arabic and cascin, approximating to T.A.C. 46.

Owing to the presence of alkali a pink colour tends to develop at the surface of the concentrate and a water layer forms on prolonged storage, but the stability is definitely improved and redispersion is much easier than with other concentrates.

It is noteworthy that on being sprayed this material does not leave a milky film on the foliage; indeed, the leaves appear scarcely to be wetted, but comparison with leaves wetted with tap water reveals a difference in colour and staining of the leaf veins quickly follows.

No definite explanation of the water-resistant qualities of the film can be offered, but it should be noted that the addition of sodium oleate to the above emulsion destroys these properties. It is possible that the effect may be due to a quicker break of the emulsion, but it is more likely that water is unable to overcome the adhesion between the film and the leaf, as both gum and cascin are non-detergents.

Skin reaction

In the course of field-plot tests in 1943 and 1944 it was noted that a number of tar derivative compounds produced a varying amount of skin reaction. The working strength of the spray was not considered likely to give trouble in this respect—at least in the case of T.A.C. 2*a* and 4, which were distributed for field tests in various parts of the country in 1944. It was found, however, that continuous exposure of the hands of the operators to the dilute spray in commercial field spraying, as was

sometimes necessary in freeing nozzles or repairing breakdowns of the machines, resulted in a number of complaints of quite serious skin irritation.

The burn produced by tar-acid compounds varies from the typical phenol type, where the skin becomes wrinkled and white, with after-effects depending on the extent of the injury, to a marked or slight discoloration, without irritation, followed by eventual desquamation.

As tar-acid compounds were being distributed for test as a 'non-corrosive' substitute for sulphuric acid it was plain that serious attention had to be paid to the skin reaction, particularly in view of the fact that the burns complained of were sometimes of a more serious character than those caused by dilute sulphuric acid. A simple test was, therefore, evolved, and any compound had subsequently to be proved relatively harmless before being passed for large-scale field trials.

Initially twelve persons were tested with several compounds, and it was found that individual susceptibility showed considerable variation. The most sensitive person, as indicated by this experiment, was selected as the subject for subsequent routine tests. The method adopted as standard was to place 1 drop of the stock emulsion on the skin of the under-side of the forearm. After 1 min. the drop was carefully wiped off with a cotton-wool swab. Notes on the skin reaction were made over a period of at least 24 hr. The use of 'neat' tar acids, prior to emulsification, for the test has been suggested because of the variation in tar-acid content of the various emulsions used (Appendix 2). In practice, however, variation of up to 15% of tar-acid content has not influenced results to a material extent. Consequently, as the 'neat' acids would never be handled by the user and as the emulsions are likely to be stabilized at 60% tar-acid content, it was considered preferable to carry out the tests as outlined. Results are given in Table 2.

Any compound giving a reaction of the type designated 'none : slight' was passed as satisfactory for large-scale field tests. Table 2 shows that both T.A.C. 2*a* and 4, distributed for large-scale tests in 1944 and concerning the irritating effects of which complaints had been received, failed to satisfy this new requirement.

For the reason stated, it is necessary, when assessing the value of a material as a haulm-killing agent, to take skin toxicity into account, therefore the combined results of field trials and skin tests have been summarized in Appendix 3.

Effectiveness and suitability

The action of tar-acid compounds is typically rapid, at least as rapid as that of sulphuric acid, discoloration and wilting of the leaves commencing within about 15 min. of application. The stems, which are usually the most difficult part of the plant to destroy, are also readily attacked. The leaves later shrivel and become light brown, and the whole haulm finally dries out as with sulphuric acid, although remaining brown.

The tar acids, the 10° C. fractions and special cuts will now be considered in the light of the data presented in Appendices 2 and 3.

TABLE 2. *Reaction of the human skin to certain tar-acid compounds*

Compound	Skin reaction: None: slight	Moderate	Severe
T.A.C. 1	—	+	—
2	—	+	—
2*a*	—	+	—
4	—	+	—
T.A.C. 15	—	+	—
16/0	—	—	+
16/1	—	—	+
16/2	—	—	+
16/3	—	—	+
16/4	—	+	—
16/5	—	+	—
16/6	—	+	—
16/7	—	+	—
16/8	+	—	—
16/9	+	—	—
T.A.C. 37*a*	—	—	+
37*b*	—	—	+
T.A.C. 18	—	—	+
19/0	—	—	+
19/1	—	—	+
19/2	—	—	+
19/3	—	+	—
19/4	+	—	—
19/5	+	—	—
19/6	+	—	—
19/7	+	—	—
19/8	+	—	—
T.A.C. 21	—	+	—
22/0	—	—	+
22/1	—	—	+
22/2	—	—	+
22/3	—	—	+
22/4	—	+	—
22/5	—	+	—
22/6	+	—	—
22/7	+	—	—
22/8	+	—	—
22/9	+	—	—
T.A.C. 42	+	—	—

Compound	Skin reaction: None: slight	Moderate	Severe
T.A.C. 25	+	—	—
26/0	—	+	—
26/2	—	+	—
26/3	+	—	—
26/4	+	—	—
26/5	+	—	—
26/6	+	—	—
26/7	+	—	—
26/8	+	—	—
26/9	+	—	—
T.A.C. 40	—	+	—
T.A.C. 27	—	—	+
33*c*	—	+	—
34*a*	+	—	—
34*c*	+	—	—
35*c*	—	+	—
36*a*	+	—	—
36*b*	+	—	—
36*c*	+	—	—
36*f*	+	—	—
46	+	—	—
T.A.C. 38	+	—	—
39/3	+	—	—
39/4	+	—	—
39/5	+	—	—
39/6	+	—	—
39/7	+	—	—
T.A.C. 41/3	—	—	+
41/4	—	+	—
41/5	—	+	—
41/6	—	+	—
41/7	+	—	—
Tar-oil winter wash	+	—	—

None: slight = no reaction or faint transient erythema.
Moderate = slight to moderate persistent erythema.
Severe = immediate, persistent erythema accompanied by sensible irritation.

Horizontal retort tar acids (*source C*). The parent material (T.A.C. 15) was almost inactive as a haulm-killing agent but all fractions (T.A.C. 16) unaccountably showed some activity, which was greatest in those boiling from 210 to 240° C. and from

280 to 290° C. The latter, however, was the only one to combine effectiveness with harmlessness to skin. Of two special cuts, T.A.C. 37*a* and 37*b*, only the latter gave a satisfactory haulm kill but both were toxic to skin, and it must be concluded that the higher of the two special cuts, T.A.C. 37*b* (260–290° C.), contained a high proportion of low-boiling material owing to the difficulty of effecting a sharp cut. This comment must undoubtedly apply to all cuts and fractions mentioned, and should be borne in mind as a possible explanation of certain discrepancies in the results.

Coke-oven tar acids (*source F*). A series of fractions (T.A.C. 41) was tested from this source. All provided a satisfactory haulm kill, but except for the highest fraction isolated, boiling from 260 to 270° C., were toxic to skin.

Vertical retort tar acids (*sources B, E and D*). One batch of tar acids obtained from source B in 1944 (T.A.C. 6) was almost completely devoid of action, and another sample from the same source in 1945 (T.A.C. 38), although slightly more active, was still unsatisfactory as a haulm-killing agent. Several fractions of T.A.C. 38 taken over the boiling range 220–270° C. (T.A.C. 39) provided a haulm kill which, although satisfactory, did not quite approach the effect of the low-temperature tar acids. All the fractions were non-toxic to skin.

A cresylic acid from source E (T.A.C. 27) was a highly efficient haulm-killing agent but was also very toxic to skin. Tar acids from the same source (T.A.C. 25) were unsatisfactory as a haulm-killing agent but non-toxic, while fractions of this boiling from 220 to 280° C. (T.A.C. 26) were both active and non-toxic. A special cut prepared subsequently, boiling from 230 to 290° C. (T.A.C. 40), was efficient but toxic to skin.

The tar acids from source D (T.A.C. 21) were unsatisfactory and harmful to skin, but fractions boiling from 230 to 290° C. (T.A.C. 22) were satisfactory haulm-killing agents and from 250 to 290° C. were also non-toxic to skin. The special cut of this range (T.A.C. 42) was satisfactory in both respects, and, in fact, gave the nearest approach to the behaviour of the low-temperature tar acids described below.

Low-temperature tar acids (*source A*). Tar acids from this source were used in the preparation of T.A.C. 1, 2, 2*a*, 4 and 18. They were successful as haulm-killing agents but had a marked toxic effect on the skin. However, when T.A.C. 18 was fractionated (T.A.C. 19), it was found that the higher fractions obtained combined effectiveness with non-toxicity to skin.

At this stage three fractions of tar acids were obtained from the same source, namely, 'XXL', high-boiling tar acid (T.A.C. 34), 'RXXL', light fraction of 'XXL' (T.A.C. 35) and 'RXXL', heavy fraction of 'XXL' (T.A.C. 36 and 46). These products, and also a further sample of the 'XL' tar acid used for T.A.C. 2 (T.A.C. 33), were made up with a variety of emulsifiers to determine the best for use in the field. T.A.C. 34 was only moderately effective but had little action on the skin, the lower boiling fraction T.A.C. 35 was good as a haulm-killing agent but was

found toxic to the skin, while the higher boiling fraction T.A.C. 36 proved satisfactory in both respects. This last-named material was, therefore, selected as the test substance for the field trials held in 1945. As previously stated, it was emulsified with a proprietary gum (T.A.C. 36*c*).

Field trials

In 1945 some thirty field trials with T.A.C. 36*c* were carried out by War Executive Committees from Northumberland to Devon. The varieties treated included Majestic, King Edward, Arran Banner, Great Scot, Redskin, Kerr's Pink and Dunbar Standard.

The results from all centres except one were described as satisfactory or fully satisfactory. The haulm kill at the one centre which reported adversely was also satisfactory, but the farmer objected that the treated haulm was tough after spraying, and not brittle as is haulm that dies naturally. The validity of such an observation is dependent on the length of time which had elapsed after spraying, and the criticism fails to take into account the substantial advantages that may accrue from chemical haulm killing.

At several centres the slightly superior action of the sulphuric acid in killing the stems was noted—another point that is well recognized.

Favourable comments on ease of handling and effectiveness were made in the reports from several centres. One example may be quoted: 'Non-corrosive T.A.C. 36*c* is much preferred to sulphuric acid. It is easy to handle. The trial gave the new fluid a rigorous test. The haulm was very tall and strong...and some rain interference was experienced, but nevertheless T.A.C. was effective.'

As in the previous season's trials with T.A.C. 2*a* reports on skin reaction varied. Most centres reported no complaints from the workmen; a few mentioned slight tingling or burning of the hands, with some peeling a few days later; there were no reports of more serious burns, as was the case with T.A.C. 2*a* in 1944.

T.A.C. 46, containing the same tar acids as T.A.C. 36*c* but incorporating the improved gum emulsifier (gum arabic + casein), and T.A.C. 42, the recommended cut from the vertical retort tar acids (source D), also with the improved gum emulsifier, became available too late in the season for general distribution. These compounds were, however, tested on a field scale at one centre against T.A.C. 36*c* and sulphuric acid. Results showed them to be, in every way, as satisfactory as T.A.C. 36*c*. In addition, the cost of the emulsifier is less, and they have the advantages previously noted of increased stability of the concentrate.

III. *Other factors involved in haulm destruction*

Haulm susceptibility

Data obtained during experiments with copper sulphate and salt in 1943 suggested that the results of all haulm-killing trials might have to be determined in the light of the physiological state of the haulm at the time of spraying. Further tests

carried out on two varieties, Doon Star and King Edward, at regular intervals throughout the 1944 season showed that T.A.C. 2, tar-oil winter wash and sulphuric acid behaved similarly, giving a highly efficient kill when the plants were young, their effect decreasing towards mid-season, then gradually increasing as haulm vigour declined, eventually reaching a high level at the end of the season when the plants were senescent. Copper sulphate and salt had very little action until the end of the season when, although the stem kill was still very poor, its effect on foliage approached that of the other sprays mentioned. The experiments were carried out at three dilution levels, 5, 10 and 15% (of stock emulsion in the case of tar derivatives), except with copper sulphate and salt, which was used throughout at 3+1% of the two substances respectively. Readings were taken 1, 3, 14 and 21 days after spraying. The results obtained with 15% spray on the variety Doon Star after 14 days are representative and are shown in Fig. 1.

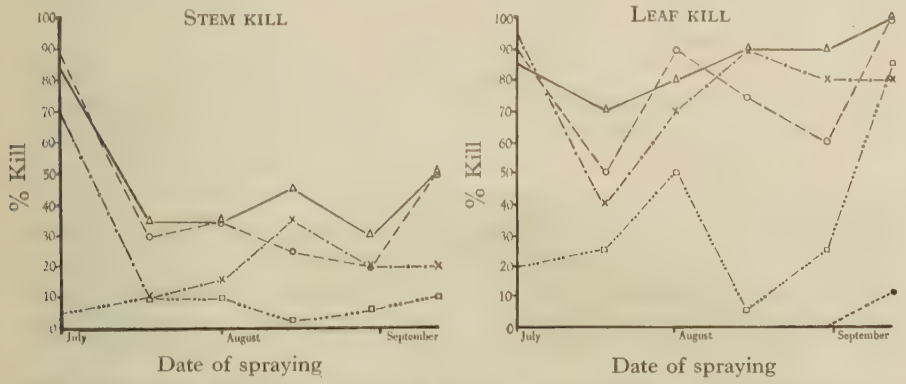

Fig. 1. Seasonal effect of haulm-killing substances. Leaf and stem kill after 14 days (var. Doon Star).

15% sulphuric acid △——△ 15% tar-distillate winter wash ×—·—·—×
15% T.A.C. 2 ○———○ 3% copper sulphate + 1% salt □—··—··□
Control (untreated) ●-----●

In view of these findings, results with all tar compounds tested are shown in Appendix 3 as a percentage of that obtained with a 10% sulphuric acid control which was invariably included in each test. By adopting this method of presentation, figures obtained at different times throughout the season can be compared. Comparative efficiency tests have been avoided very late in the season, as at that time relatively inefficient compounds such as copper sulphate and salt may become nearly as efficient, at least in leaf kill, as sulphuric acid.

The implications of these findings require careful study in relation to practical requirements. As was stated earlier, haulm-killing compounds are needed for use late in the season as a routine measure against the blight infection present in many crops at that time. For this purpose it is satisfactory to use weaker concentrations or less efficient compounds than would be required if severe blight commenced

earlier and it became necessary to destroy the haulm while still vigorous and green. In the same way the destruction of haulm in a relatively vigorous state for seed production requires a most efficient spray. References to 'late season' are thus meant to apply more to haulm vigour than actual date; the condition would be reached earlier in time in the case of an early variety and later in the case of a late-maturing variety. If a general purpose spray is required then the efficiency requirement is high, but if the intention is merely to destroy yellowing senescent haulm a highly efficient spray is not so necessary. Under the latter conditions even copper sulphate and salt might prove satisfactory.

In a trial of the effect of T.A.C. 2*a* on the haulm of fifteen commercial varieties some differences were observed, but the plants varied greatly in initial vigour and it is probable that the results were dependent on varietal differences in maturity.

Observations during the trials showed that inefficient haulm killing, as well as permitting second growth of the haulm itself, may result in second growth of the tubers, a most undesirable condition.

At one time it was thought that tar-oil winter wash, because of its slow action, inhibited second growth of a vigorous haulm to a greater extent than tar acids. As winter wash contains considerable amounts of neutral oils, mixtures of neutral oils and tar acids were tried (T.A.C. 28–32, 43–45). These proved either ineffective or no more active than the equivalent amount of the tar-acid constituent applied alone. The bronzing effect produced by the tar-oil winter wash was not very noticeable in using these mixtures. In view of the fact that the more efficient tar-acid compounds have since proved quite as effective as tar-oil winter wash in preventing second growth of vigorous haulm, further work on this material was discontinued.

Susceptibility to haulm-killing sprays appears to vary from plant to plant, but it was found impossible to obtain any reliable figures to illustrate this. Nevertheless, it should be borne in mind as a possible explanation of the patchy kill sometimes obtained, for which no other obvious reason can be found. Laboratory experiments on cut leaves were carried out to determine the relative susceptibility of (1) leaflets from the same leaf and (2) leaves from different positions on the same plant. Seven leaflets were removed from each of ten leaves taken at random and dipped in 10% of stock emulsion of T.A.C. 36*c*. The amount of discoloration was noted after 30 min. The greatest variation from the mean in the case of leaflets from the same leaf was $\pm 3{\cdot}6\%$. Leaflets from leaves at the top, centre and bottom of a number of plants were similarly treated and the time taken to produce 90% discoloration noted. The results of a representative experiment are given in Table 3.

TABLE 3. *Susceptibility of leaves from different parts of the plant to action of* 10% *of stock emulsion of T.A.C.* 36*c*

Position of leaves on plant	Time taken to produce 90% discoloration (min.)
Top	10
Centre	15
Bottom	25

The figures indicate that resistance increases with the age of the leaf. No comparable data are, however, available to show whether, late in the season, there is a falling off in resistance of the lower leaves as compared with the centre leaves, marking the onset of senescence.

The evidence presented above indicates the difficulties which must be faced in conducting haulm-killing trials and very probably provides an explanation of the discrepancies in the results of tests of the same compound by different workers. The factor of haulm vigour must, therefore, be given careful attention in any future trials.

It was noted in several of the large-scale field trials that the kill produced in the rows between the tractor wheelings was much more satisfactory than in those rows over which the spray boom alone had passed. This effect was noted irrespective of the compound used and was not produced where the sprayer was horse drawn, thus indicating a mechanical bruising effect of the body of the tractor. It was decided in view of this observation, in one trial, to use a drag consisting of a bar of wood immediately in front of the spray boom during the application of 15% sulphuric acid. The resulting kill was noticeably superior to that obtained where the drag was not used.

Weather conditions

Weather is a factor of considerable practical importance in haulm destruction, since spraying must frequently be carried out when conditions are very unsettled. This applies particularly to seasons when blight is developing rapidly during prolonged spells of wet weather. Several tests were carried out in order to examine the influence of rain on the kill obtained with certain haulm-killing compounds. The method employed was to apply 1 gal. water, by means of a watering can, at intervals after spraying, to six-plant plots which had been treated respectively with T.A.C. 2*a*, tar-oil winter wash, copper sulphate and salt and sulphuric acid. The eventual kills obtained are shown in Table 4. Readings were also taken after 14 days, but these are not given owing to the complication introduced by second growth of the haulm on certain of the plots.

TABLE 4. *Influence of artificial rain on extent of kill obtained with certain haulm-killing compounds. Var. Doon Star. Date 9 August* 1944. *Percentage leaf:stem kill after 7 days*

	Time of application of 1 gal. water					
Compound	Immediate	1 hr.	3 hr.	6 hr.	24 hr.	Control: no water
T.A.C. 2*a* (10% stock emulsion)	10:5	65:20	65:20	60:15	55:15	60:20
Tar-oil winter wash (10% stock emulsion)	2:2	20:5	60:5	50:5	65:5	55:5
Copper sulphate 3% + salt 1%	2:0	2:0	2:0	5:0	10:0	15:2
Sulphuric acid 10%	5:2	60:10	65:20	90:30	80:30	90:30

The water appeared to have no effect on the final kill given by T.A.C. 2*a* if it was applied 1 hr. or more after application of the spray. The action of tar-oil winter

wash was affected by water applied up to 3 hr. after application of the spray. The kill obtained with sulphuric acid was, however, considerably reduced up to 6 hr. after application, while the effect of copper sulphate and salt was greatly diminished by water applied even 24 hr. later. These results indicate the possibility that water-soluble compounds may, in general, tend to be washed off or diluted by rain to the detriment of their haulm-killing effect. Obviously an ideal compound would be capable of application during rain or at least would not be adversely affected by subsequent rainfall. It should be borne in mind when examining the results set out in Table 4 that the 1 gal. water used, while being equivalent in amount to $\frac{1}{6}$ in. of rain was not really comparable owing to the extreme rapidity of application. It should also be noted that the kill obtained with T.A.C. 2*a* in the control was much less satisfactory than that normally obtained with this compound. This experiment was carried out relatively early in the season when differences between effective and non-effective applications could be the more easily detected, but another experiment was carried out late in the season during rain which continued for 12 hr. after application (Table 5).

TABLE 5. *Influence of rain on the kill obtained with certain haulm-killing compounds. Var. Majestic. Date* 30 *August* 1945. *Percentage leaf:stem kill at successive dates*

	Days after application of spray			
Compound	1	5	7	14
T.A.C. 36*c* (5% tar acid)	45:0	70:5	80:5	95:50
T.A.C. 46 (5% tar acid)	40:0	75:5	80:5	98:75
Sulphuric acid, 10%	0:0	10:0	30:0	95:45
Control (unsprayed)	0:0	0:0	5:0	10:0

In this experiment the influence of rain on the effect of sulphuric acid up to 1 week after application is very apparent, while the tar acids were relatively resistant to washing off. It is of interest to note that both T.A.C. 36*c* and T.A.C. 46 contained a gum emulsifier, considered likely to improve retention of the spray by the haulm (see p. 11). After 14 days the kill on the sulphuric acid plots greatly improved, and it is probable that this tendency to equality in the effect of all compounds was due to the general senescence of the foliage which set in at this point as shown by the figures for the unsprayed control. This would tend to cause the rapid death of haulm weakened but not obviously damaged by the spray treatment. Moreover, blight was fairly severe and tended to mask the final differences.

This high degree of retention of tar-acid compounds under adverse conditions by the haulm is a valuable asset, and further tests designed to compare the effects of different emulsifiers were carried out in the laboratory on wet and dry leaves. The tar-acid compounds were used in dilutions containing 5% tar acids and sulphuric acid was used in a 10% solution. Three medium-sized Majestic leaflets were dipped per treatment. The results are shown in Table 6.

TABLE 6. *Comparison of effect of certain haulm-killing compounds on leaves of the same age washed in water before and after treatment. Time taken (min.) for at least 90% discoloration of leaflets*

Treatment	Compound			
	T.A.C. 36*a*	T.A.C. 36*c*	T.A.C. 36*e*	H_2SO_4
Dipped	30	15	30	90
Washed and dipped	30	18	39	120
Dipped and washed	*	26	75	*
Washed, dipped and washed	*	55	*	*

* No discoloration occurred.

The three compounds, T.A.C. 36*a*, *c* and *e*, differ only in the emulsifier, *a* containing S.R. + sodium oleate, *c* a proprietary gum and *e* sodium caseinate. It is obvious that the gum emulsion gave the most satisfactory results, which possibility was suggested in the section on emulsifiers (p. 11).

The action of sulphuric acid is shown in Table 6 to be considerably slower than that of any of the tar acids used, and on wet leaves its effect is reduced still further. When the leaves were washed subsequent to treatment no action by sulphuric acid was apparent, a tendency which was observed in the field tests. It would appear possible, therefore, to apply tar acids in gum emulsion successfully to wet leaves or under weather conditions where the kill given by sulphuric acid would be greatly diminished.

Occasionally on hot days the leaves of potato plants tend to wilt and become flaccid. Tests using tar acids were carried out on leaflets allowed to become flaccid and on turgid leaflets. Although some evidence was obtained that turgid leaves became discoloured slightly more rapidly, indicating that the toxic compound might be entering through the open stomata, evidence from further experiments was inconclusive. Initial experiments on the effect of temperature alone on the rapidity of leaf kill by tar acids also gave inconclusive results.

Control of blight

Six $\frac{1}{100}$-acre plots of King Edward were sprayed, each with a different compound, 1 month before lifting. Random samples of tubers from these and from an unsprayed control plot were stored in sacks for 8 months and then examined. No difference whatever was observed in the degree of sprouting in tubers from the different treatments. All treatments, on the other hand, appeared to provide a certain degree of control of blight (Table 7).

In further tests, random samples of tubers from two 6 × 6 Latin square experiments, one of Majestic, the other of King Edward, were lifted 1 month after spraying. These were stored in sacks and examined 4 months later (Table 8). The reduction in blighted tubers attributable to treatment was significant except in the case of copper sulphate and salt and tar-oil winter wash, both on the variety Majestic, but

there was no significant difference between the various spray treatments. Observations made at the time suggest that the amount of blight which developed in the tubers was inversely proportional to the haulm kill, which was unfortunately poor throughout for unknown reasons.

TABLE 7. *Control of blight in tubers—comparison of effects of six haulm-killing compounds applied* 31 *August* 1943

Compound	Strength of spray %	Blighted tubers %
Sulphuric acid	10	0·76
T.A.C. 2	5*	1·63
Tar-oil winter wash	7*	1·36
Copper sulphate + salt	3 + 3	3·95
Copper sulphate + sulphuric acid	3 + 2·5	3·12
Copper chloride	3	3·78
Control (unsprayed)	—	7·33

* Of stock emulsion.

TABLE 8. *Control of blight—comparison of effects of five haulm-killing compounds applied* 14 *September* 1944

Compound	Strength of spray %	Blighted tubers % (mean of six plots)	
		Majestic	King Edward
Sulphuric acid	10	1·45	6·98
T.A.C. 2*a*	10*	0·92	8·27
T.A.C. 4	10*	2·32	8·49
Tar-oil winter wash	10*	2·21	10·24
Copper sulphate + salt	3 + 1	3·45	9·43
Control (unsprayed)	—	4·17	14·81

* Of stock emulsion.

Experiments and observations have shown that when only one side of a leaflet is sprayed, as frequently happens in the field, sporulation of the blight fungus tends to continue on the other side until the whole leaflet is killed. Thus tar-oil winter wash, owing to its slow action, permits this sporulation to continue for a much longer period after spraying than do the rapid-acting compounds, a point which may be of considerable importance. Other experiments showed that blight spores were killed by contact with any of the above compounds. The blight fungus can continue to spore on any green areas of the stem untouched by spray fluid, thus emphasizing the desirability of a rapid and effective kill of stems as well as leaves.

'Setting' of skin of tubers

It has been claimed that where haulm destruction is practised, the skins of the tubers are more 'set' when lifted than would otherwise be the case, and that in consequence they sustain less damage during lifting, thus reducing wastage due to

invasion of wounds by parasitic organisms. A number of experiments designed to obtain data on this subject were carried out. Two methods of assessing the degree of 'setting' of the skin were tried.

(1) Physical resistance to damage was tested by holding the tuber in the hand and rubbing the skin sharply three times in succession with the ball of the thumb. Tests were carried out by the same worker throughout and the pressure applied was as uniform as possible. Tubers where the skin tore at the first rub were awarded 0 points, at the second 1 point, at the third 2 points and where no tearing occurred 3 points. Tubers for test were first washed to eliminate the abrasive action of the soil and then dried, as moisture reduces friction between the thumb and the skin of the tuber.

(2) As 'setting' progresses, the thickness and degree of suberization of the skin increases; this, it was presumed, might affect the extent of water loss from the flesh. In an attempt to eliminate the personal factor involved in the rubbing test, tubers were weighed before and after subjection for 48 hr. to a temperature of 35–40° C. Similarly, tubers were weighed before and after storage at 20° C., for 1 week, over concentrated sulphuric acid. Although these weight-loss tests gave promising results in the first experiment they failed subsequently to show differences clearly demonstrated by the rubbing test and were, therefore, discontinued. The data given were obtained by the latter method.

The first experiment was on a very small scale, tests being carried out on six tubers (var. Majestic) from each treatment. The tubers used were taken from the bulked produce of each plot and were of approximately uniform size. Table 9 shows that 50 days after spraying there appeared to be a relation between the state of the haulm and the degree of skin 'setting' of the tubers. When sprayed on 9 August the haulms were in a vigorous condition.

TABLE 9. *Degree of 'setting' of skin in Majestic tubers lifted on 28 September 1943 from plots sprayed with haulm-killing compounds on 9 August 1943*

Compound	% leaf:stem dead on lifting	Skin set marking (max. = 100)
Sulphuric acid 10%	100:100	100
T.A.C. 2 (10% of stock emulsion)	100:100	100
Tar-oil winter wash (10% of stock emulsion)	100:100	100
Cupric chloride 5%	100:100	100
Copper sulphate 3% + salt 3%	100:65	83
Control—unsprayed	70:5	22

The following year a larger experiment was carried out in order to obtain data on the progress of skin 'setting' during a 50-day period. Plots of the variety Doon Star were sprayed at intervals, commencing again on 9 August when the haulms were in full vigour. Tubers were lifted on 28 September, 50 days after the first spraying date. Skin tests were made on the entire bulked produce of three plants in each case. The results of this experiment are given in Table 10.

TABLE 10. *Degree of 'setting' of skin in Doon Star tubers lifted on 28 September 1944 from plots sprayed at intervals from 9 August 1944*

Compound	Sprayed 17 days before lifting		Sprayed 30 days before lifting		Sprayed 45 days before lifting		Sprayed 50 days before lifting	
	% leaf:stem dead on lifting	Skin set marking (max.= 100)	% leaf:stem dead on lifting	Skin set marking (max.= 100)	% leaf:stem dead on lifting	Skin set marking (max.= 100)	% leaf:stem dead on lifting	Skin set marking (max.= 100)
Sulphuric acid 10%	100:95	87	100:100	87	100:100	100	100:60	67
T.A.C. 2*a* (10% of stock emulsion)	100:95	20	100:80	40	100:100	100	100:75	100
Tar-oil winter wash (10% of stock emulsion)	95:30	40	100:100	60	100:100	100	100:100	80
Copper sulphate 3% +salt 1%	95:25	0	85:20	47	35:25	60	95:90	100
Control—unsprayed	25:5	10	25:5	10	25:5	10	25:5	10

The figures for the state of the haulm do not indicate the rapidity of kill obtained with the various compounds. As results in general indicate that 'setting' of the skin is accelerated by the death of the haulm, the length of time for which the haulm has been nearly or completely dead is a factor of obvious importance. This is well illustrated by the comparison of the degree of skin 'setting' obtained by spraying with sulphuric acid and tar-oil winter wash, which are fast- and slow-acting compounds respectively. The discrepancy in the results obtained with tubers from plots sprayed with the rapid-acting T.A.C. 2*a*, 17 and 30 days respectively before lifting, is difficult to explain but may indicate that despite the apparent rapidity of discoloration and shrinkage of the stems obtained with this compound they are not killed as rapidly as is the case with sulphuric acid. The figures suggest that the rapidity and extent of stem kill obtained, whether second growth occurs or not, has considerable influence on skin 'setting'; thus the desirability of a compound having a marked action on stems as well as leaves is further accentuated.

A further series of tests was carried out on the 6×6 Latin square experiments (var. Majestic and King Edward) referred to in the section on blight control. As stated, the haulm kill obtained was poor throughout, and no difference was found in either case in the degree of skin 'setting' between tubers from any of the sprayed plots and those from the unsprayed control plots during the month which elapsed between spraying and lifting and for 1 month thereafter, although progressive 'setting' was demonstrated in all treatments throughout the period of the experiment.

The results of these experiments suggest that haulm destruction accelerates the 'setting' of the skins of the tubers to what is probably a material extent, from the point of resistance to mechanical injury, only if a really satisfactory and rapid kill of both stems and leaves is obtained. Data obtained from control plots and various field observations suggest that skin 'settings' during maturation of the haulm is

a gradual process, accelerating during senescence and death. The time of spraying in relation to the physiological state of the haulm is, therefore, probably of considerable importance in determining the extent of the beneficial skin 'setting' effect obtained at lifting. Further, the longer, within reason, that it is possible to leave the crop in the ground after spraying, the greater will be the degree of skin 'setting' when lifted. Additional work is, however, necessary before the full effects of varietal, seasonal and other interacting factors can be fully appreciated.

Cooking quality of the tubers

Comprehensive tests were carried out at the Ministry of Food's Experimental Kitchen on random samples from the produce of each of thirty-six plots of a Latin Square experiment (var. Majestic) where plots were sprayed with T.A.C. 2*a*, T.A.C. 4, sulphuric acid, tar-oil winter wash and copper sulphate and salt. No differences in flavour, texture or colour of the flesh attributable to treatment were found.

Residual effects on soil

No deleterious effect on the soil of sulphuric acid, T.A.C. 2, tar-oil winter wash, copper chloride and copper sulphate and salt at strengths commonly used in haulm destruction was indicated by the germination and growth of wheat, peas and lettuce sown in samples of top soil taken 7 weeks after spraying. No noticeable effect was observed in sugar beet following potatoes which had been sprayed with the above compounds, nor in winter wheat following potatoes sprayed with T.A.C. 36*c*, 42 and 46, sodium chlorate (3%) and sulphuric acid respectively. In these tests the haulm was of normal size and gave good coverage to the soil. Where haulm is small obviously more spray will reach the ground.

Discussion

The haulm-killing sprays which must be considered either in the light of the results presented in this paper or on account of their present commercial use are: (1) sulphuric acid, (2) di-nitro-cresol compounds, (3) cupric chloride, (4) copper nitrate, (5) copper sulphate and salt, (6) sodium chlorate, (7) calcium cyanamide, (8) tar-oil winter wash and (9) tar-acid compounds.

Before discussing the merits and disadvantages of these various compounds it would be of interest to summarize briefly the qualities which, in the opinion of the authors, should be present in an ideal haulm-killing spray. These are: (1) efficient kill of both leaves and stems even when the haulm is in a relatively vigorous state, (2) rapid action, (3) no deleterious residual effects on subsequent crops, (4) no deleterious effect on quality of tubers, (5) harmless to operators, (6) absence of other qualities objectionable to operators, (7) no deleterious effect on clothing, (8) no corrosive action on metal, (9) low cost, (10) ease of transport, (11) ease of mixing with water, (12) stability of concentrate (keeping quality), (13) effectiveness when applied in wet weather.

Sulphuric acid fulfils eight of the above requirements (1, 2, 3, 4, 6, 9, 11, 12). One of the main advantages in the use of this compound, apart from the satisfactory kill obtained, is the low cost of material which, using the standard commercial application of 100 gal. of 15% Brown Oil of Vitriol, is approximately 14*s*. per acre. Transport of concentrated acid is inconvenient and expensive owing to the necessary use of glass carboys or other special containers, but the main disadvantage is the corrosive nature of the liquid, both to operators and their clothing and to spraying machinery. When comparing costs of this and other sprays both the high initial cost of acid-resistant sprayers and protective clothing and the considerable annual depreciation in their capital value must be taken into consideration. One commercial user of sulphuric acid expressed the opinion that four seasons is the maximum life of an acid-resistant spraying machine. Despite these most serious disadvantages, sulphuric acid remains probably the best all-round spray for use by contractors and others accustomed to it. The bulk of the potato acreage in this country is, however, in the hands of small growers who in the main cannot be provided for under any scheme of contracting and who cannot themselves undertake spraying with this compound. It is for this reason that so many efforts have been made to find a satisfactory non-corrosive spray.

Di-nitro-cresol compounds have not been found satisfactory, although they may be reasonably effective late in the season on senescent haulm. Owing to their objectionable staining properties, if for no other reason, they are unlikely to gain any popularity.

Copper chloride may be regarded as fairly effective, with a moderate speed of action. The crystalline compound is markedly hygroscopic, which is a slight disadvantage. Applied at 3% the cost of a 100 gal./acre application would not be below 30*s*. for material. Copper nitrate, which is comparable in effect, would cost approximately the same.

Copper sulphate and salt has relatively little effect except when applied to senescent haulm, and even then a rather poor kill of stems is obtained. However, the compound is cheap, the cost of materials per acre for a 3 + 1% solution applied at 100 gal. being approximately 13*s*. In addition, the constituents are generally available and the spray is not unpleasant to use.

The copper compounds mentioned have a deleterious action on some metals such as brass which, although relatively slight, should not be overlooked. In addition, copper sprays must not be placed in iron containers as some of the copper is removed by the iron.

Sodium chlorate is a moderately effective agent for killing leaves, but unfortunately, like copper sulphate and salt, it has relatively little effect on the stems, unless possibly when the haulm is senescent. Even when applied in a 3% solution at a rate of 100 gal./acre, the maximum strength advocated, the cost is low. In our experiments the substance has been almost as effective at half that strength, the cost for material then being about 10*s*. per acre only. Sodium chlorate suffers from the disadvantage of inflammability, and care must be taken with any clothing, etc.,

upon which the spray has fallen. Preliminary observations indicate that even when applied at 100 gal. of 3% spray per acre there are no grounds for any fear of toxic residual effects in the soil.

The limited tests carried out with calcium cyanamide do not suggest that this compound has any great value as a haulm-killing agent.

There seems to be a certain divergence of opinion as to the degree of stem kill essential. In the opinion of the authors, on the score of blight control, acceleration of skin 'setting' in the tubers and removal of mechanical obstructions to harvesting, the maximum possible stem kill is desirable.

When the haulm is senescent and, therefore, easily killed, one of the less efficient but cheaper compounds such as copper sulphate and salt or sodium chlorate may be adequate, but if, for the purpose of destroying relatively vigorous haulm, a spray is required which more nearly approaches sulphuric acid in general effectiveness, tar-derivative compounds must be considered.

Tar-oil winter wash is a moderately effective killing agent and fulfils all other requirements except those of rapid kill and low cost. The disadvantages of a slow kill from the point of view of blight control have already been discussed. The cost of material per acre for the 7% (of stock emulsion) strength of spray recommended, applied at the rate of 100 gal./acre, is approximately 35*s*.

The tar-acid compounds which can be recommended at the moment under the code numbers T.A.C. 42 and T.A.C. 46 fulfil all requirements except that of low cost. The present cost of materials, which must be regarded as provisional, is approximately 40*s*. per acre for a 10% (of 60% stock emulsion) spray applied at the rate of 100 gal./acre. Although the use of these compounds is barely an economic proposition at that figure, they are the only sprays tested which approach closely to the level of effectiveness of sulphuric acid, and as mentioned above, capital outlay on and depreciation of equipment as well as the comfort of the operators must be taken into account when comparing costs.

An additional point in favour of the tar-derivative compounds tested is that their action appears to be only slightly impaired by rain occurring either before, during or after the spraying operation, a quality enhanced by the gum emulsifier used in T.A.C. 42 and T.A.C. 56. The effectiveness of sulphuric acid and other water-soluble compounds tested tends, on the other hand, to be reduced considerably under these circumstances. This property may be of great importance in some seasons. Further, if blight is present in a crop, a prolonged spell of wet weather may make it most desirable to kill the haulm immediately on account of the rapidity of spread of the disease under humid conditions.

Even at the present cost, comments made by those using experimental consignments for large-scale trials indicate that there might be a limited demand for these compounds.

It is evident that there is present in some tar acids a certain constituent, or more likely a group of constituents, which is active as a haulm-killing agent and is non-toxic to skin. It is possible that further work, using the refined methods of

fractionation now available, combined with biological testing, will throw light on the nature of these substances and possibly lead to the development of even more effective non-corrosive haulm-killing sprays.

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APPENDIX 1

List of substances tested in haulm-killing trials

A. *Substances giving unsatisfactory kill*

(1) *Sprays*

Acetic acid 10 %
Calcium hypochlorite (filtered soln.) 5 %
Copper acetate 5 %
Creosote (light, medium and heavy) 5 %
Ferrous sulphate 5 %
Hydrochloric acid 10 %
Sodium chloride 3 %
Sodium sulphite 5 %
Sulphurous acid (7·5 % SO_2 w/w) 5 %
Potassium dichromate 5 %
Calcium chloride 5 %
Chlorine water (8·5 % chlorine w/w) 5 %
Copper sulphate 5 %
Ferric chloride 5 %
Formalin 12·5 %
Magnesium chloride 5 %
Sodium hypochlorite (15 % available chlorine) 5 %
Phenol 5 %
Potassium permanganate 5 %
Certain tar-acid compounds (T.A.C.) (details in text)
Certain tar-base compounds (T.B.C.) (details in text)
Certain tar-oil compounds (T.O.C.) (details in text)

(2) *Dusts*

Bleaching powder 60 % in talc, 4 cwt./acre
Copper sulphate 12 % + salt 12 % in talc, 2 cwt./acre
Cupric chloride 12 % in talc, 2 cwt./acre.

B. *Mixtures of substances no more satisfactory than same strength of one of the constituents*

Copper sulphate 5 % + acetic acid 5 %
Copper sulphate 5 % + hydrochloric acid 2·5 %
Cupric chloride 3 % + salt 3 %
Cupric chloride 3 % + hydrochloric acid 2·5 %
Cupric chloride 3 % + acetic acid 5 %
Ferrous sulphate 5 % + salt 5 %
Ferrous sulphate 5 % + sulphuric acid 2·5 %
Ferrous sulphate 5 % + acetic acid 5 %
Potassium dichromate 5 % + sulphuric acid 1 %

C. *Substances considered promising (details in text)*

(1) *Sprays*

Ammonium thiocyanate 5 %
Copper nitrate 3 %
Copper sulphate 3 % + sulphuric acid 2·5 %
Sodium di-nitro-ortho-cresylate 2 % + ammonium sulphate 2 %
Sodium hydroxide 10 %
Tar-oil winter wash 7 %
Copper chloride 3 %
Copper sulphate 3 % + salt 1 %
Sodium chlorate 3 %
Sulphuric acid 10 %
Certain tar-acid compounds (T.A.C.)

(2) *Dusts*

Calcium cyanamide ½ cwt./acre.

APPENDIX 2

Composition of tar acid and certain other tar derivative sprays tested

Code no. of compound	Supplier	Carbonization process	Description of material	Composition of emulsion: Tar acids or oils (%)	Added neut. oil	Emulsifier	% Water
T.A.C. 1	A	L.T.	XL tar acids	76·4	—	Sod. oleate	6·2
T.A.C. 2	A	L.T.	XL purified tar acids	76·4	—	Sod. oleate	6·2
T.A.C. 2*a*	A	L.T.	XL purified tar acids	60·0	—	Perminal E.M.L. + casein	33·0
T.A.C. 3	A	L.T.	Crude-tar oil (first 30 % of wax oil)	60·5	—	Perminal E.M.L. + casein	32·0
T.A.C. 4	A	L.T.	Crude-tar acids b.p. 230–280° C.	60·2	—	Perminal E.M.L. + casein	37·3
T.A.C. 5	B	V.R.	Tar distillate	61·25	—	Perminal E.M.L. + casein	35·18
T.A.C. 6	B	V.R.	High-boiling tar acids	60·0	—	Perminal E.M.L. + casein	37·3
T.A.C. 7	C	H.T.	Medium creosote	50·9	—	Perminal E.M.L. + casein	44·7
T.A.C. 8	A	L.T.	Alkaline extract	—	—	Water miscible	—
T.A.C. 9	A	L.T.	Sulphuric acid waste product	—	—	Water miscible	—
T.A.C. 10	A	L.T.	Tar acids from first 30 % of heavy wax oil	56·5	—	Sod. oleate	33·9
T.O.C. 11	A	L.T.	Light hydrocarbon oil	—	—	—	—
T.O.C. 14	C	H.T.	Tar oil (13 % tar acids)	50·0	—	Sod. oleate	41·0
T.A.C. 15	C	H.T.	Whole-tar acids	50·0	—	Sod. oleate	41·0
T.A.C. 16 (series)	C	H.T.	10° C. fractions tar acids 190–290° C.	50·0	—	Sod. oleate	41·0
T.O.C. 17	A	L.T.	Tar oil (46 % tar acids)	50·0	—	Sod. oleate	41·0
T.A.C. 18	A	L.T.	Whole-tar acids	50·0	—	Sod. oleate	41·0
T.A.C. 19 (series)	A	L.T.	10° C. fractions tar acids 190–280° C.	50·0	—	Sod. oleate	41·0
T.O.C. 20	D	V.R.	Tar oil	50·0	—	Sod. oleate	41·0
T.A.C. 21	D	V.R.	Whole-tar acids	50·0	—	Sod. oleate	41·0
T.A.C. 22 (series)	D	V.R.	10° C. fractions tar acids 190–290° C.	50·0	—	Sod. oleate	41·0
T.B.C. 23	E	V.R.	Crude distilled pyridine bases	—	—	Water miscible	—
T.B.C. 24	E	V.R.	Residue of steam-distilled bases	50·0	—	Sod. oleate	41·0
T.A.C. 25	E	V.R.	Crude carbolic ex vertical tar oil	50·0	—	Sod. oleate	41·0
T.A.C. 26 (series)	E	V.R.	10° C. fractions tar acids 190–290° C.	50·0	—	Sod. oleate	41·0
T.A.C. 27	E	V.R.	Cresylic acid	50·0	—	Sod. oleate	41·0
T.A.C. 28 (series)	A	L.T.	XL tar acids + anthracene oil	30–50	5·8–30	Turkey red oil	—
T.A.C. 29 (series)	A	L.T.	XL tar acids + anthracene oil	40–80	8·0–40	Sod. oleate + S.R.	0·7–1·6
T.A.C. 30 (*a* series)	A	L.T.	XL tar acids + anthracene oil	40–66·7	6·7–26·6	Sulphite lye	—
T.A.C. 30 (*b* series)	A	L.T.	XL tar acids + anthracene oil	40–66·7	6·7–26·6	Sod. oleate + sul. lye	—

L.T. = low-temperature carbonization.
H.T. = high-temperature carbonization.
V.R. = vertical retort carbonization.
C.O. = coke-oven carbonization.

APPENDIX 2 (*continued*)

Code no. of compound	Supplier	Carbonization process	Description of material	Composition of emulsion: Tar acids or oils (%)	Added neut. oil	Emulsifier	% Water
T.A.C. 31 (series)	A	L.T.	XL tar acids + anthracene oil	48–80	8–32	Sod. oleate + rosin + S.R.	3·2
T.A.C. 32 (series)	A	L.T.	XL tar acids + anthracene oil	47–78	0–31	Amm. oleate + S.R.	4·0
T.A.C. 33*a*	A	L.T.	XL purified tar acids	80·0		Sod. oleate + S.R.	4·0
T.A.C. 33*b*	A	L.T.	XL purified tar acids	50·0		S.R.	45·0
T.A.C. 33*c*	A	L.T.	XL purified tar acids	60·0		Proprietary gum	37·0
T.A.C. 34*a*	A	L.T.	XXL high-boiling tar acids	80·0		Sod. oleate + S.R.	4·0
T.A.C. 34*b*	A	L.T.	XXL high-boiling tar acids	50·0		S.R.	45·0
T.A.C. 34*c*	A	L.T.	XXL high-boiling tar acids	60·0		Proprietary gum	37·0
T.A.C. 35*a*	A	L.T.	RXXL light high-boiling tar acids	80·0		Sod. oleate + S.R.	4·0
T.A.C. 35*b*	A	L.T.	RXXL light high-boiling tar acids	50·0		S.R.	45·0
T.A.C. 35*c*	A	L.T.	RXXL light high-boiling tar acids	60·0		Proprietary gum	37·0
T.A.C. 35*d*	A	L.T.	RXXL light high-boiling tar acids	90·0		Sod. oleate + S.R.	3·0
T.A.C. 36*a*	A	L.T.	RXXL heavy high-boiling tar acids	80·0		Sod. oleate + S.R.	4·0
T.A.C. 36*b*	A	L.T.	RXXL heavy high-boiling tar acids	50·0		S.R.	45·0
T.A.C. 36*c*	A	L.T.	RXXL heavy high-boiling tar acids	60·0		Proprietary gum	37·0
T.A.C. 36*e*	A	L.T.	RXXL heavy high-boiling tar acids	60·0		Sod. caseinate	38·9
T.A.C. 36*f*	A	L.T.	RXXL heavy high-boiling tar acids	60·0		Prop. gum + casein	37·9
T.A.C. 36*g*	A	L.T.	RXXL heavy high-boiling tar acids	60·0		Prop. gum + casein	38·5
T.A.C. 37*a*	C	H.T.	Tar acids b.p. 210–240° C.	60·0		Proprietary gum	37·0
T.A.C. 37*b*	C	H.T.	Tar acids b.p. 260–290° C.	60·0		Proprietary gum	37·0
T.A.C. 38	B	V.R.	Whole-tar acids	60·0		Proprietary gum	37·0
T.A.C. 39 (series)	B	V.R.	10° C. fractions tar acids 220–270° C.	60·0		Proprietary gum	37·0
T.A.C. 40	E	V.R.	Tar acids b.p. 230–290° C. (ex. creosote oil)	60·0		Proprietary gum	37·0
T.A.C. 41 (series)	F	C.O.	10° C. fractions tar acids 220–270° C.	60·0		Proprietary gum	37·0
T.A.C. 42	D	V.R.	Tar acids b.p. 230–290° C.	60·0		Proprietary gum	37·0
T.A.C. 43 (series)	A	L.T.	RXXL heavy high-boiling tar acids + light oil	30–54	6–30	Gum arabic + casein	38·4
T.A.C. 44 (series)	A	L.T.	RXXL heavy high-boiling tar acids + middle oil	30–54	6–30	Gum arabic + casein	38·4
T.A.C. 45 (series)	A	L.T.	RXXL heavy high-boiling tar acids + creosote	30–54	6–30	Gum arabic + casein	38·4
T.A.C. 46	A	L.T.	RXXL heavy high-boiling tar acids	60·0		Gum arabic + casein	38·4

L.T. = low-temperature carbonization.
H.T. = high-temperature carbonization.
V.R. = vertical retort carbonization.
C.O. = coke-oven carbonization.

EFFECTIVENESS AND SUITABILITY OF TAR-ACID COMPOUNDS

A. *Main compounds*

Compound	No. of tests	Leaf:stem kill after 14 days as % kill of 10% H_2SO_4 0–9	10–49	50–69	70–89	90 and above	Satisfactory leaf:stem kill	Relatively harmless to skin
1943*								
T.A.C. 1	2	.	.	.	.	LS	+	−
2	5	.	.	.	.	LS	+	−
Tar-oil winter wash (7%)	5	.	.	S	L	.	+	+
1944†								
T.A.C. 2	10	.	.	.	LS	.	+	−
2*a*	1	.	.	S	L	.	+	−
3	1	.	LS	.	.	.	−	.
4	7	.	.	S	L	.	+	−
5	1	.	LS	.	.	.	−	.
6	1	LS	.	.	.	.	−	.
7	1	LS	.	.	.	.	−	.
8	1	LS	.	.	.	.	−	.
9	1	LS	.	.	.	.	−	.
10	1	LS	.	.	.	.	−	.
T.O.C. 11	1	LS	.	.	.	.	−	.
14	1	S	.	L	.	.	−	.
T.A.C. 15	1	LS	.	.	.	.	−	.
T.O.C. 17	1	.	S	L	.	.	−	.
T.A.C. 18	1	.	.	.	.	LS	+	−
T.O.C. 20	1	.	LS	.	.	.	−	.
T.A.C. 21	1	.	.	LS	.	.	−	.
T.B.C. 23	1	LS	.	.	.	.	−	.
24	1	LS	.	.	.	.	−	.
T.A.C. 25	1	.	S	.	L	.	−	.
27	1	.	.	.	S	L	+	−
Tar-oil winter wash	5	.	S	.	L	.	−	.
1945‡								
T.A.C. 2*a*§	8	.	.	LS	.	.	−	.
33*a*	3	.	.	.	.	LS	+	−
33*b*	3	.	S	L	.	.	−	.
33*c*	3	.	.	S	L	.	+	−
34*a*	3	.	.	.	S	L	+	+
34*b*	3	.	S	L	.	.	−	.
34*c*	3	.	LS	.	.	.	−	.
35*a*	3	.	.	.	S	L	+	−
35*b*	3	.	S	.	L	.	−	.
35*c*	3	.	.	S	L	.	+	−
35*d*	3	.	.	.	S	L	+	−
36*a*	3	.	.	.	LS	.	+	+
36*b*	3	.	.	S	L	.	+	+
36*c*	12	.	.	.	LS	.	+	+
36*e*	3	.	S	.	L	.	−	.
36*f*	3	.	.	S	L	.	+	+
36*g*	3	.	.	LS	.	.	−	.
37*a*	2	.	S	.	L	.	−	.
37*b*	2	.	.	.	S	L	+	−
38	2	.	S	L	.	.	−	.
40	2	.	.	.	.	LS	+	−
42	2	.	.	.	.	LS	+	+
46	3	.	.	.	S	L	+	+
Tar-oil winter wash	3	.	S	.	L	.	−	.

L = leaf; S = stem.

* Tests in 1943 carried out on var. Majestic using 5% of stock emulsion except where otherwise noted.
‡ Tests in 1944 carried out on var. Doon Star using 10% of stock emulsion.
‡ Tests in 1945 carried out on var. Majestic using 5% of tar acids except where otherwise noted.
§ 1944 stock emulsion used, therefore deterioration possible cause of reduced kill.

APPENDIX 3 (*continued*)

B. *Fractions*

Compound	No. of tests	Leaf:stem kill after 14 days as % kill of 10% H_2SO_4					Satisfactory leaf:stem kill	Relatively harmless to skin	Satisfactory in both respects
		0–9	10–49	50–69	70–89	90 and above			
T.A.C. 15	1	LS	.	.	.	.	–	–	–
16/0	1	.	LS	.	.	.	–	–	–
16/1	1	.	LS	.	.	.	–	–	–
16/2	1	.	.	S	L	.	+	–	–
16/3	1	.	.	S	L	.	+	–	–
16/4	1	.	.	S	L	.	+	–	–
16/5	1	.	S	L	.	.	–	–	–
16/6	1	.	S	L	.	.	–	–	–
16/7	1	.	S	L	.	.	–	–	–
16/8	1	.	LS	.	.	.	–	+	–
16/9	1	.	.	S	L	.	+	+	+
T.A.C. 37*a*	2	.	S	.	L	.	–	–	–
37*b*	2	.	.	.	S	L	+	–	–
T.A.C. 18	1	.	.	.	.	LS	+	–	–
19/0	1	.	.	.	LS	.	+	–	–
19/1	1	.	.	.	LS	.	+	–	–
19/2	1	.	.	S	L	.	+	–	–
19/3	1	.	.	.	S	L	+	–	–
19/4	1	.	.	.	.	LS	+	+	+
19/5	1	.	.	.	.	LS	+	+	+
19/6	1	.	.	.	.	LS	+	+	+
19/7	1	.	.	.	.	LS	+	+	+
19/8	1	.	.	S	L	.	+	+	+
T.A.C. 38	2	.	S	L	.	.	–	+	–
39/3	2	.	.	.	S	L	+	+	+
39/4	2	.	.	.	S	L	+	+	+
39/5	2	.	S	.	L	.	–	+	–
39/6	2	.	.	S	L	.	+	+	+
39/7	2	.	.	.	S	L	+	+	+
T.A.C. 21	1	.	.	LS	.	.	–	–	–
22/0	1	.	.	LS	.	.	–	–	–
22/1	1	.	.	LS	.	.	–	–	–
22/2	1	.	S	L	.	.	–	–	–
22/3	1	.	S	L	.	.	–	–	–
22/4	1	.	.	.	S	L	+	–	–
22/5	1	.	.	.	S	L	+	–	–
22/6	1	.	.	.	S	L	+	+	+
22/7	1	.	.	S	L	.	+	+	+
22/8	1	.	.	S	L	.	+	+	+
22/9	1	.	.	S	.	L	+	+	+
T.A.C. 42	2	.	.	.	.	LS	+	+	+
T.A.C. 25	1	.	S	.	L	.	–	+	–
26/0	1	.	S	L	.	.	–	–	–
26/2	1	.	S	.	.	L	–	–	–
26/3	1	.	.	.	S	L	+	+	+
26/4	1	.	.	.	S	L	+	+	+
26/5	1	.	.	S	.	L	+	+	+
26/6	1	.	.	.	S	L	+	+	+
26/7	1	.	.	S	.	L	+	+	+
26/8	1	.	.	S	L	.	+	+	+
26/9	1	.	S	.	.	L	–	+	–
T.A.C. 40	2	.	.	.	.	LS	+	–	–
T.A.C. 41/3	2	.	.	.	S	L	+	–	–
41/4	2	.	.	S	L	.	+	–	–
41/5	2	.	.	.	LS	.	+	–	–
41/6	2	.	.	.	.	LS	+	–	–
41/7	2	.	.	.	S	L	+	+	+

L = leaf; S = stem.

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ADDENDUM

Since going to press, information has been received from North America that a compound developed there recently, in which the active constituent is di-nitro-ortho secondary butyl phenol, has proved more effective for potato haulm destruction than the di-nitro-cresol derivatives previously used. In addition it has been learned that considerable trouble has been experienced there, in certain areas, with 'stem-end browning' and vascular discoloration of tubers, attributed to premature destruction of the haulm. No such phenomenon has been observed in Britain.

EXPERIMENTS WITH SOILS USED FOR CASING BEDS OF THE CULTIVATED MUSHROOM, *PSALLIOTA CAMPESTRIS*

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In the present paper an account is given of observations and experiments on the influence of the casing soil on the cropping of beds of the cultivated mushroom, *Psalliota campestris*. It is shown from the results of a survey of cropping at thirty-nine mushroom farms that when the compost and cultural conditions are suitable for cropping, the production of mushrooms may be largely determined by the soil used for casing. Casing soils are classified into four grades depending on the numbers of sporophores formed, their rate of growth and the type of mushroom produced. A laboratory method of assessing the cropping potentialities of soils is described, and examples are given of its use for investigating field factors affecting the selection of soils for casing and the nature of the soil factors affecting cropping. It is suggested that chemical substances or specific surfaces in soils may promote cropping, but that close contact with air is necessary for them to be effective. It is concluded that direct trial is the only reliable method of assessing the value of a material for casing and that considerable fundamental research is needed on casing soils.

It is a remarkable fact that mushroom beds must be covered with a layer or casing of 'soil' to bring them into bearing. Without the casing soil few or no mushrooms are produced. The manner in which cropping is brought about by the casing soil has been the subject of considerable speculation. Lambert (1938), in an interesting review of mushroom problems, records the general view that the formation of sporophores is largely a result of the relative poverty of the casing soil in nutrients as compared with the compost. Experimental evidence in support of this contention is, however, lacking, and the subject is greatly in need of investigation.

An interesting fact is that materials of diverse nature and origin may make excellent casing soils when carefully managed and used in suitable surroundings. A wide range of surface soils and subsoils is in common use, and in favourable situations geological materials such as chalk, gypsum or sandstone rubble and even heavy unweathered clay have proved successful. Certain physical qualities are required in casing materials, e.g. the properties of taking up water readily and losing it slowly, remaining in a porous, granular condition when watered repeatedly, and settling with the compost as the latter shrinks during cropping. When a casing material lacks any of these properties considerable improvement in behaviour and cropping may be brought about by adding coarse sand, fine gravel or peat. Liming of acid materials is necessary and disease organisms must be absent. When all these conditions are satisfied, however, considerable differences in yield of mushrooms occur. In 1938 the authors visited thirty-nine mushroom farms where cultural conditions were good and beds were well run with mycelium and found that yields

varied from $\frac{1}{2}$ lb. to more than 3 lb. of mushrooms per sq.ft. of bed. At six places the yield was from 3 to 4 lb./sq.ft., at fifteen it was from 2 to $2\frac{3}{4}$ lb., at thirteen it was from 1 to $1\frac{3}{4}$ lb., and at five it was $\frac{3}{4}$ lb. or less per sq.ft. At one farm, beds were found to be cropping slowly and producing small light mushrooms except on a small section of one bed where the mushrooms were heavy and crowded together. The 'run' of mycelium in the compost was uniformly good and the casing soil was similar throughout in texture, condition and *p*H value. It was found that the casing soil had been dug from a small grass field after removing the turf layer, but the soil in the good section had been taken from a different part of the field from the main bulk of soil. No evidence of disease was found in any of the beds.

This striking example of the apparent influence of the casing soil on cropping, shape and weight of mushroom led to the experimental work described in the present paper. The work was interrupted in 1939 by the outbreak of war and is unlikely to be continued, but the results obtained appeared to be worth recording.

Laboratory method of studying casing soils

As facilities for carrying out experiments in mushroom houses were not available, a method was developed of growing mushrooms on a small scale in the laboratory. Wood (1937) had shown in a study of 'damping off' of cultivated mushrooms that they could be grown in glass troughs, 25 cm. diam. and 10 cm. deep, on 6 cm. of pressed moist pure-culture spawn covered with 2–2·5 cm. ($\frac{3}{4}$–1 in.) of soil. Troughs of this size were much too large for our purpose, since comparisons of ten to twenty soils in duplicate or triplicate were visualized, and only by keeping the total area covered by the experiment small could uniformity in temperature and humidity conditions be obtained. Fortunately, it was found that beakers could be used instead of troughs. It is possible to grow mushrooms in beakers as small as 250 ml. capacity, but for comparative purposes the size should not be less than 600 ml. (7·5 cm. diam. and 14·5 cm. tall) or the soil will dry out too readily and be difficult to keep in a uniformly moist condition.

When the properties of casing soils are being compared it is desirable to eliminate as many other variables as possible. The use of pure-culture moist spawn reduces variability in the growing medium to a minimum. All parts of the medium are of proved suitability for growth of the mushroom mycelium and can be made similar in biological age, in vigour of the mycelium and in physical and chemical properties. The usual medium for the preparation of spawn is composted horse manure in a short well-composted granular condition. This is pressed into quart milk bottles, sterilized by steam under pressure and inoculated with a culture obtained from spores of a selected mushroom. When ready for use as spawn, the contents of the bottles are fully 'run' with mycelium in active, vigorous condition. They are not, however, entirely uniform since parts have been longer 'run' than others, but after removal from the bottles the contents can be crumbled readily and thoroughly

mixed together. Such material is free from diseases and pests, and, as was pointed out by Wood, cropping occurs in a comparatively short time after casing—in his experiments in about 3 weeks as compared with 2 months when compost is used—thus reducing 'errors' of management and the possibilities of contamination. Provided care is taken in pressing the spawn into beakers, close similarity can be obtained in depth, compactness, temperature, aeration and humidity, except as these properties may be subsequently modified by the casing soil. The enclosed conditions, particularly near the bottom of the beaker, do not appear to have any adverse effect when the casing soil crops satisfactorily, but if little or no cropping occurs degeneration of the spawn with production of a yellow liquid occurs. This point may be of interest to growers who are concerned about the apparent lack of aeration of the bottom layers in mushroom beds.

The technique found most suitable was as follows:

Freshly prepared fully run mushroom spawn, obtained from the same original culture, was removed from its bottles and crumbled into small fragments by hand and thoroughly mixed together. Sufficient was prepared for the number of experiments to be carried out. Spawn of a white variety of mushroom was used, as this is said to be less vigorous than spawn of brown varieties and is the more commonly grown.

For each experiment a layer $2\frac{1}{2}$ in. deep was pressed firmly into the bottom of a 600 ml. tall form, lipless, Pyrex beaker (3 in. diam. and $5\frac{3}{4}$ in. tall). The casing soil to be examined was air-dried, ground in a wooden mortar to pass a 3 mm. round-holed sieve and thoroughly mixed together. The soil was moistened with distilled water to bring it to the sticky point and then carefully worked into a granular condition. It was then placed on top of the spawn in the beakers and tamped down gently but firmly with a flat-headed glass rod until a layer 1 in. in depth had been built up free from large air spaces. The beakers were covered with $3\frac{1}{2}$ in. diam. watch-glasses supported on rubber cushions (short lengths of rubber tubing slit lengthwise and slipped over the rim of the beaker) to allow entry and exit of air. They were placed in a room at 70° F. for a few days until the mycelium had joined up or grown afresh in the spawn and was showing signs of entering the soil, after which they were kept in a room at 55–60° F. The soil was watered as necessary, usually every other day, but once the mycelium had grown thoroughly into the soil, water was absorbed very slowly (this may be a cause of variability in mushroom beds).

Sporophores generally appear in the soil within 10 days and may continue to form for many weeks. With a soil that crops well the experiment is usually brought to an end by disturbance and loss of soil in removing mushrooms. Triplicate experiments agree very closely and there is usually no need to have more than duplicates. Finer grinding of the soil to pass 2 or 1 mm. sieves has no effect on growth or cropping.

In only one experiment was disease found to be carried by the casing soil, and this experiment was rejected from all comparisons. It is believed by the authors that in all other instances the soils were free from disease or known competitive organisms.

Comparison of cropping properties of soils in beakers and in mushroom houses

For this purpose, samples of soil were collected from the thirty-nine places already visited, and prepared as described in the previous section. Before being used, texture, *p*H value, available potassium and available phosphorus were determined. Each soil was examined in duplicate by the beaker method and observations made of the character of hyphal growth in the soil, the time of appearance of sporophores, their relative abundance and rate of growth. In all but five instances the results in beakers agreed qualitatively with the results in mushroom houses. The exceptions were soils that had been heat sterilized immediately before use. In beakers these soils become permeated and covered with a dense weft of hyphae in which very few sporophores formed or grew beyond pin-head size. This condition only developed in mushroom houses in depressions in the beds where the soil was continually moist, and it appears that better control of aeration and humidity is necessary for the examination of these soils in beakers than was possible for us to obtain.

Classification of casing soils

The soils examined fell fairly readily into four distinct classes as follows:

Class I. The soil is rapidly and uniformly invaded by extremely fine hyphae which cover the soil particles with a smoke-like growth of variable density. At an early stage numerous rather coarse strands appear throughout the soil, and the surface becomes studded with sporophores. The latter form at all depths but are mainly on the surface and grow rapidly into fine heavy mushrooms. Cropping is almost continuous, and in mushroom houses high yields of 3–4 lb./sq.ft. are obtained. In beakers it is necessary to remove mushrooms when they are small buttons about $\frac{1}{2}$ in. across, and yields have, therefore, very little significance. The first buttons are usually removed from beakers after 23–26 days and from seven to nine buttons may be removed in 42 days. The number of sporophores reaching picking size is usually a good indication of the cropping properties of the soil, but at times considerable disturbance of the soil is caused by picking, resulting in breaking of feeding strands. As a rule it is safer to assess the class to which a soil belongs from the number, distribution, condition and rate of growth of the sporophores.

Class II. Soils in this class produce yields of 2–$2\frac{3}{4}$ lb./sq.ft. The mycelium enters the soil fairly rapidly. Hyphae are plentiful, fine in character and uniformly distributed, but are more numerous in the lower part of the soil. Strands are numerous but relatively fine in character. Few or many sporophores are produced usually in groups either below or in the surface of the soil. They grow less rapidly than in class I soils, and cropping occurs in definite flushes. Similar features are shown in beakers. Sporophores are much less numerous than in class I and usually take a day or two longer to reach picking size. As a rule four to six buttons are picked in 42 days from setting up the beakers.

Class III. Soils in this class produce yields of 1–1¾ lb./sq.ft. The mycelium enters the soil comparatively slowly, usually at definite points, from which fine hyphae grow upwards in a fanwise manner. A very variable number of strands is produced which are not much thicker than hyphae and are usually difficult to distinguish from them. Sporophores are few in number and are produced in groups. They grow comparatively slowly, and cropping occurs in flushes of moderate size with fairly long intervals between. Growth in beakers is similar. Very few sporophores are produced. They usually occur in one or two groups from which they grow to button size singly. The first one is ready for picking in about the same time as in class II and is usually followed at long intervals by not more than two others. The buttons are much less fleshy than in classes I and II.

Class IV. Soils in this class produce yields of ¾ lb. or less per sq.ft. Growth is similar to that in class III but fewer sporophores are produced. They are small in size and grow very slowly into light mushrooms. After a small first flush the beds practically cease cropping. In beakers one mushroom of poor type is usually obtained but there may be none.

It is apparent from the above results that the casing soil is a determining factor in cropping and may account to a large extent for the big differences in yield that growers obtain. If the results are typical of conditions generally, then approximately 15·5% of the casing soils used by growers are capable of producing 3 lb. or more of mushrooms per sq.ft. of bed, 38·5% are capable of producing 2–2¾ lb., 33% 1–1¾ lb., and 13% produce ¾ lb. or less. High and low yields are apparently not uncommon, and the average yield appears to be about 1¾ lb./sq.ft.

In the soils examined, texture, *p*H value, available potassium and available phosphate appeared to have no influence on the result apart from two soils with *p*H values of 5·4. Excluding these two soils the ranges of the above soil properties were as follows:

	Class I	Class II	Class III	Class IV
Texture	Silty loam to clay loam	Sandy loam to clay	Fine sandy loam to clay	Very fine sandy loam to clay loam
*p*H	6·4–7·3	6·1–7·6	5·7–7·6	6·2–7·2
Av. K	Very low to very high	Very low to very high	Very low to high	Very low to medium
Av. P	Low to medium high	Low to medium	Low to medium high	Low to medium

Addition of precipitated chalk to the soils with *p*H 5·4 improved cropping slightly in one instance from class IV to class III, and in the other from class III to class II. Liming did not, however, produce a first class soil, and it appeared to have no effect on less acid soils.

Although the amounts of nitrogen and organic matter in the soils were not determined, wide variation in each class occurred, for each included soils and subsoils of widely different textures.

It seems, therefore, from these investigations that it is not sufficient to assess a soil for casing purposes from its structure, texture and *p*H value as is commonly done.

These properties only serve to eliminate soils that are undesirable in physical qualities. They bear no relation to the inherent cropping properties of the soil, and in using them alone a first class soil may be rejected in favour of one that has not the same property of cropping heavily.

Influence of soil profile and site on cropping

Many growers in this country dig soil for casing from the field in which their mushroom houses stand, usually working progressively across the field from a single site. If the soil is uniform, similar results are likely to be obtained from year to year. More often than not, however, the soil varies in a number of important respects—in depth down to the unweathered material, in the depth of each horizon, in drainage and quite commonly in type. Old pastures show variations in composition due to differences in the herbage and to the habits of stock.

These facts are little known by growers. Soil horizons and sometimes unweathered geological material are dug out, mixed together and used for casing. Since soil horizons and unweathered geological material differ from one another biologically, chemically and physically, it is possible that they also differ in cropping properties. Much of the variation in cropping that growers obtain may therefore be due to indiscriminate mixing of soil horizons with different cropping properties or to differences in the soil profile in parts of the field from which the soil is obtained. The following examples show that the subject merits thorough investigation:

Example	Origin of soil	Soil horizon (in.)	Depth of freely drained soil (in.)	Texture	*p*H	Arable = A Grassland = G	Cropping class by beaker test
1	Sedentary						
	Weald Clay	0–5	5	C.L.	6·5	G	I
		5–9		C.L.	6·5		I
2	Sedentary						
	Weald Clay	0–6	0	Z.L.	6·4	G	III
		6–12		C.L.	7·6		III
3	Drift over						
	Weald Clay	0–6	6	V.F.S.L.	5·7	G	III
		6–12		V.F.S.L.	6·2		IV
4	Sedentary						
	Ashdown Sand	2–9	9	V.F.S.L.	6·3	G	II–
		9–16		V.F.S.L.	6·3		III
5	Sedentary						
	Ashdown Sand	2–9	9	V.F.S.L.	6·1	G	II
		9–16		V.F.S.L.	6·1		II–
6	Sedentary						
	Folkestone Beds	0–7	> 36	F.S.L.	5·8	A	III
		7–14		S.L.	5·8		IV
7	Sedentary						
	Keuper Marl	0–6	6	S.C.L.	6·5	G	II–
		6–12		C.L.	6·7		II
		12–30		Marl + Clay	7·0		IV
		30–42		Red Clay	7·6		II

Texture abbreviations: S.L. = sandy loam. Z.L. = silt loam.
F.S.L. = fine sandy loam. S.C.L. = sandy clay loam.
V.F.S.L. = very fine sandy loam. C.L. = clay loam.

Example 1 is an instance of a first class cropping soil dug from sedentary Weald Clay to a depth of 9 in. The lower 5–9 in. showed evidence of periodic waterlogging.

Example 2 is a similar soil to 1, but is waterlogged in wet weather to the surface. The soil was dug to a depth of 12 in. for casing. The top 6 in. contained more organic matter and was more granular than the second 6 in. but cropped similarly. It is a poor cropping soil compared with 1. Possibly waterlogging by its influence on the herbage and organic matter of the soil has something to do with this, although it apparently has no influence on the second horizon in example 1.

Example 3 was taken from a higher lying part of the same field as 2, where the clay was covered with about 18 in. of alluvium and the drainage was free to 6 in. Although much lighter in texture and better drained, the top horizon is no better than in 2, and the second horizon is much worse.

Examples 4 *and* 5 are from two sites in the same field. The soil profile, texture and *p*H values were similar in both instances, but the soils cropped quite differently as the grower found. It was the usual practice to remove the top 2 in. of turf and dig out the next 14 in. Site 5 was much better than 4, and beaker tests indicated that this was chiefly due to the poor cropping properties of the second horizon of site 4, and to a certain extent to the better cropping properties of the top horizon of site 5.

Example 6 is a well-drained arable soil that is physically very suitable for casing mushroom beds. It was, however, a very poor cropping soil with the second horizon much poorer than the first. It was slightly acid, but cropping was not improved by liming.

Example 7 is a very interesting instance in which the grower obtained apparently unaccountable variations in cropping from year to year or from house to house. The site where the soil was obtained was the face of a cutting into a sloping field. At the face of the cutting the sequence of horizons was as indicated, but at the sides the horizons came to the surface in turn. On looking into past practices, it was found that poor crops were obtained when soil was taken from the sides where the marl plus clay came sufficiently near to the surface to be reached by digging. Beaker tests of each horizon showed that the marl plus clay was quite useless for cropping, whereas the soil above and the geological material below were moderately good.

These results indicate that the cropping properties of soils may vary considerably with depth and with position in the same field, that soils physically alike may crop very differently and that it is impossible from physical examination alone to determine whether a soil is suitable for casing. There is a suggestion that the upper horizons are better than the lower ones, in which case the rejection of the top 6 in. of grassland—as is commonly done to avoid soil-borne diseases—may involve rejection of the best cropping soil. On the other hand, deep horizons have been found to be good. The geological material in example 6 is moderately good and the writers know of Kimmeridge Clay dug from 60 ft. below the surface that was excellent. As is well known, geological material consisting largely of gypsum or chalk is used

to case beds in the quarries around Paris, and material from the Thanet Beds has been used successfully in the caves at Chislehurst, Kent.

Experiments on the cropping properties of soils

For these experiments three soils with contrasting cropping properties were used. The soils were:

Lab. no.	Cropping class	Texture	*p*H	Origin
26	I	Z.L.	7·0	Not known. Supplied by contractor
13	IV	Z.L.	6·2	Thin alluvium over Weald Clay. Poorly drained. Taken at 2–10 in. Under grass
31	IV	F.S.C.L.	7·2	Not known. Supplied by contractor

The object of the experiments was to ascertain the nature of the factors affecting cropping, whether they were physical, chemical or biological in nature. Two series of experiments were carried out. In the first series, the casing soil consisted of two layers formed by different soils. In the second series of experiments different soils were mixed together. All experiments were carried out in duplicate in beakers as previously described, except that the total depth of soil was $1\frac{1}{2}$ in., consisting in the first series of experiments of $\frac{3}{4}$ in. of one soil and $\frac{3}{4}$ in. of the other soil.

First series

(I) *Effect of placing soil* 26 *on top of soil* 13. This is a combination of a class I cropping soil on top and a class IV soil underneath. The result of this experiment was most unexpected. As regards cropping, the combination behaved as a class I soil. The layer of class IV soil next to the spawn apparently had no effect on cropping. Sporophores formed rapidly in the top soil but not in the lower soil. Four fleshy buttons were removed 4 weeks after starting the experiment and six more were removed in the following 4 weeks. The spawn underneath had by then shrunk considerably in depth and the surface soil was broken up as a result of removing the mushroom buttons. The experiment was stopped at this stage and the layers of soil examined. The top soil was covered with healthy sporophores the size of mustard seed and was filled with thick strands which were continuous with much finer strands in the lower soil. Growth in the lower soil was quite sparse in comparison with the top soil and sporophores were absent. It seems remarkable that the few very fine hyphae in the bottom soil were able to conduct nutrients to the strands in the top soil at a sufficiently rapid rate.

(II) *Effect of placing soil* 13 *on top of soil* 26. This combination is the reverse of (I) and the result was again unexpected. No sporophores were formed. The lower soil became filled with many strands of medium thickness and a few that were very thick.

The strands continued into the top soil where they became much thinner and fewer in number. The top soil apparently determined the cropping and prevented the formation of sporophores in the lower soil. No shrinkage of the spawn occurred as in experiment 1, due presumably to the absence of cropping.

(III) *Effect of placing soil* 26 *on top of soil* 31. This combination was inferior to 26/13 in cropping capacity. Sporophores were much less numerous in the surface soil and only half the crop was removed in the same time. When the soils were broken up, a dense growth of fine hyphae and a few medium strands were found in 31. A clear cut boundary existed between 31 and 26 and only medium strands were found in 26. No sporophores were found in 31. Apparently the lower soil had lowered the cropping capacity of the top soil.

(IV) *Effect of placing soil* 31 *on top of soil* 26. This combination is the reverse of (III) and similar to (II). As in (II) no sporophores were formed. When broken apart after 2 months, soil 26 was filled with numerous medium strands with no sporophores. The change from soil 26 to 31 was clear cut and marked by branching of most of the strands into a dense growth of fine hyphae. As in (I) the cropping capacity of soil 26 was completely repressed by soil 31.

(V) *Effect of placing soil* 13 *on top of soil* 31. These are both class IV soils producing different types of mycelial growth, that in 13 being sparse and fine with very few strands and that in 31 being plentiful and fine with many fine strands. Both types produce few sporophores. In the present experiment the type of mycelial growth in each soil was characteristic of that soil, and the change in passing from one soil to the other was clear cut. No sporophores were produced in 31 but a few were produced on the surface of 13. These mostly failed to develop. Two grew very slowly and reached the size of a pea 6 weeks after the experiment started. Apparently 31 improved the cropping properties of 13 slightly.

(VI) *Effect of placing soil* 31 *on top of soil* 13. These soils again retained their growth characteristics but no sporophores were produced.

Second series

In these experiments mixtures of two soils were used consisting of 195 parts by weight of one soil to 5 parts by weight of the other soil, e.g. 195 parts of 26 were mixed with 5 parts of 13, denoted thus 26_{13}. The mixtures used were 26_{13}, 26_{31}, 31_{13}, and 31_{26}.

(VII) *Mixture* 26_{13}. Compared with 26 alone, mycelial growth was less vigorous and uneven, fewer strands were produced and fewer sporophores and the latter were unevenly distributed. Only half the crop was obtained.

(VIII) *Mixture* 26_{31}. Compared with 26 alone, more fine hyphae were produced, but strand formation, production of sporophores and cropping were no different.

(IX) *Mixture* 31_{13}. No difference from 31 alone.

(X) *Mixture* 31_{26}. No difference from 31 alone.

A plausible explanation of these results may be given if it be assumed that (*a*) the

thickening of fine hyphae into strands and the formation of sporophores are separate and distinct phases in cropping; (*b*) the formation of fine hyphae and the formation of strands in soils are promoted by opposing factors, either one of which may outweigh the other; (*c*) the formation of sporophores on the strands requires contact of the surface of the soil with air, or contact of the strands with air; and (*d*) factors adverse to mycelial growth may occur in soils.

For example, the outstanding facts regarding soils 26 and 13 are:

(1) The cropping of 26 is not affected when 13 is underneath it.

(2) Sporophores do not form in 26 when 13 is above it.

(3) The types of mycelial growth in the two soils are distinctly different and are not affected by contact of the soils or passage of the mycelium through one soil into the other.

(4) Mixing a small quantity of 13 with 26 alters the type of mycelial growth and manner of formation of sporophores and considerably reduces cropping.

These facts may be explained by assuming that (*a*) factors promoting strand formation preponderate in 26, (*b*) factors adverse to mycelial growth preponderate in 13 and are relatively more effective than the strand-forming factors in 26, and (*c*) contact of the soil or strands with air is required for the formation of sporophores.

Considering the two soils 26 and 31, the chief facts are:

(1) The cropping of soil 26 is reduced when 31 is underneath it.

(2) Sporophores do not form in 26 when 31 is above it.

(3) Mycelial growth in 31 consists largely of a dense growth of fine hyphae and in 26 of a dense growth of strands. When 26 is above 31, a proportion of fine hyphae of 31 continues into 26 and there is a reduction in the number of strands in 26. When 31 is above 26, the strands in 26 divide at the boundary of the two soils and continue into 31 as fine hyphae.

(4) Mixing a small quantity of 31 with 26 has very little effect. More fine hyphae are produced but production of sporophores and cropping are not affected.

(5) Mixing a small quantity of 26 with 31 has no effect on the type of growth in 31 or on cropping.

These facts may be explained by assuming that (*a*) factors promoting strand formation preponderate in 26; (*b*) factors promoting the formation of fine hyphae preponderate in 31, and their effect on the strand-promoting factors in 26 depends on the relative amounts of 26 and 31 in contact or mixed together; and (*c*) contact of the soil or strands with air is required for the formation of sporophores.

The lack of adverse effect of 13 on the development of fine hyphae in mixture 31_{13} may indicate that the chief adverse effect of 13 is on the formation of strands.

It is only possible from these experiments to speculate on the nature of the factors affecting cropping. A point of great interest is the apparent lack of mobility of the factors in soils 26 and 13, no transfer from one soil to another taking place when the soils are placed in contact. Mixing of the soils is necessary for the properties of one to affect the other. This seems to exclude direct biological influences on cropping

unless the organisms concerned have very specific soil requirements. Residues from biological activity may, however, be concerned, and these would appear to be strongly held by the soil and require close contact with air to be effective. They may occur as single chemical substances insoluble in water or as complex substances with specific surface action.

The factors in soil 31 have a slight influence on soils 26 and 13 when 31 is in contact with them. The effect is largely apparent as a change in type of mycelial growth and may therefore be due to a nutrient that is taken up from soil 31 and moved forward as the hyphae grow.

Experiments designed to test these speculations were planned but were not carried out owing to the outbreak of war. It is hoped, however, that others will be interested in the experiments described and have facilities for repeating and extending them.

In other instances, other factors than the ones supposed may affect cropping. The chief value of the experiments is that they show that the casing soil has a controlling influence on cropping, and that in the present state of our knowledge the only method of assessing the value of a casing soil is by small-scale experiments in boxes or in beakers as has been described.

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OBSERVATIONS ON THE PATHOGENICITY OF *CALONECTRIA RIGIDIUSCULA* (BERK. & BR.) SACC. ON *THEOBROMA CACAO* L.

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(With Plates 1 and 2)

Calonectria rigidiuscula has been found associated with an acute and a chronic form of dieback of cacao. It can also infect cankers caused primarily by *Phytophthora palmivora*, and the lesions following attack by the capsids *Sahlbergella singularis* and *Distantiella theobroma*. The association with the capsid lesions is of great economic importance since it appears that capsids alone are capable of killing only green shoots, and that the severe damage caused to woody shoots follows *C. rigidiuscula* infection of the capsid lesions. Acute dieback occurs only in certain districts after exceptional drought and may be considered primarily the result of environmental conditions. The main importance of chronic dieback is that it prevents the recovery of cacao which has been seriously weakened from other causes. *C. rigidiuscula* infection retards the healing of *P. palmivora* cankers and also produces a lesion in the xylem tissue which may be extensive.

C. rigidiuscula has been established in wounded cacao stems of all ages without difficulty. The spread of the fungus is slow, but is more rapid in the xylem than in the cortical tissues. Spread is greatest in the unlignified tissues.

Introduction

Infection by *Calonectria rigidiuscula* (Berk. & Br.) Sacc. forms one component of a complex of four disorders to which cacao (*Theobroma cacao* L.) in West Africa is subject. These are canker (caused primarily by *Phytophthora palmivora* (Britt.) Butl.), injuries by the capsids *Sahlbergella singularis* Hagl. and *Distantiella theobroma* Dist., acute dieback, and chronic dieback. To elucidate the degree of damage ascribable to *C. rigidiuscula* infection, it has proved necessary in this paper to follow the description of the fungus with brief accounts of these four disorders. The pathogenicity of *C. rigidiuscula* is then considered, with special reference to the association of the fungus with capsid injury, canker and dieback.

The data recorded here have been collected in the Gold Coast, but recently the fungus has been cultured also from capsid lesions, canker and chronic dieback of cacao in Nigeria.

Description of *Calonectria rigidiuscula*

C. rigidiuscula has a world-wide distribution and has been recorded on a number of different hosts (Wollenweber & Reinking, 1935); this description is, however, confined to the fungus on cacao. The fungus has been recorded on cacao in a number

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of different countries, and has been associated particularly with canker. Early work, which has been summarized by Petch (1910), attributed canker to *C. ceremea* Zimm. in Ceylon, and *C. flavida* Mass. in the West Indies. Both these are considered to be synonymous with *C. rigidiuscula* (Wollenweber & Reinking, 1935). Both Carruthers in Ceylon and Howard in the West Indies claimed to have produced cankers by inoculation with the spores of this fungus, but their technique has been criticized on the grounds that the spores were not taken from pure culture and the inoculations were therefore liable to contamination (Petch, 1910). Canker has also been attributed to *Spicaria colorans* van Hall-de Jonge in Surinam (de Jonge, 1909) and to *Fusarium decemcellulare* Brick in the Cameroons (Brick, 1908): these are both imperfect states of *C. rigidiuscula* (Wollenweber & Reinking, 1935). Neither de Jonge nor Brick claimed positive results with inoculations. In the absence of conclusive evidence of pathogenicity *C. rigidiuscula* was considered merely to be an early saprophyte following closely on *Phytophthora palmivora*, the primary cause of canker. During the present investigations typical canker symptoms have never been produced by inoculation with *C. rigidiuscula*.

Certain fungi have been recorded as associated with capsid lesions in the Gold Coast (Dudgeon, 1910) and in the Belgian Congo (Mayné, 1917). It is of particular interest that a *Fusarium* sp. was mentioned among other fungi recorded by Mayné in the Belgian Congo.

Cultural characteristics

The spores formed earliest in the development of the fungus are the conidia whose appearance corresponds closely to the published description of *F. decemcellulare* (Wollenweber & Reinking, 1935). The microconidia are formed first and appear in damp weather on the surface of freshly affected tissue, especially infected capsid lesions on green stems. At this stage the fungus forms a whitish bloom on the surface of the lesion. The microconidia are normally single-celled or occasionally 1-septate, $8 \times 4\,\mu$, borne in chains usually coiled into tight heads on simple or sparingly branched conidiophores, occurring freely over the surface of the mycelium. Macroconidia appear occasionally with the microconidia, but are usually found on dead tissues of woody stems following either dieback or infected capsid attack, or on the surface of dead trunks; the sporodochia are short, pale pinkish buff (Ridgway, 1912), emerging through cracks in the bark, close together and regularly arranged, giving a distinctive speckling to the stem. The macroconidia are thin, hyaline, sickle-shaped, with a distinct pedicel and cream-coloured in the mass, usually 9- or 10-septate, but the septation appears to vary with different conditions, and in some collections the spores were predominantly 6- and 7-septate. The following measurements were recorded for the macroconidia: 5-septate, $64 \times 7\,\mu$; 7-septate, $71 \times 8\,\mu$; 9-septate, $76 \times 8\,\mu$. The identification of the conidial material was confirmed by the Imperial Mycological Institute by comparison with other specimens of *F. decemcellulare*. The perithecia of *C. rigidiuscula* are found only on long dead tissues; they are pale pinkish buff (Ridgway, 1912), closely aggregated

into groups, and borne on the surface of small stromata which are practically buried in the host tissues. These stromata emerge through cracks in the bark in much the same way as the sporodochia; the ascospores are 3-septate, hyaline, slightly curved and rounded at both ends, $27 \times 7\ \mu$; the asci are club-shaped and normally 4-spored. Single ascospore isolations of this fungus yielded typical cultures of *F. decemcellulare*, and this in conjunction with the published description (Wollenweber & Reinking, 1935) leaves no doubt that the fungus is in fact *C. rigidiuscula*.

C. rigidiuscula grows readily on artificial media. Two agar media were used: cassava dextrose, and Brown's synthetic (Brown, 1925). Growth was luxuriant on both and a compacted mat of mycelium was formed. On cassava dextrose agar the mycelium varied between white and Corinthian purple (Ridgway, 1912), and the agar below the mycelium was stained the same colour. There was practically no coloration on Brown's agar. The colour of the mycelium was characteristic of the isolate and was retained after subculturing and also after passage through cacao, thus providing a useful check on the inoculation technique. Microconidia as found in the field were formed freely after about 3 days' growth at tropical temperatures. The macroconidia were formed after 2–3 weeks at about 27° C. in slimy yellow pionnotes. In some cultures the whole surface was slimy with many small pionnotes, while in others only one or two large ones were produced. Cultures on sterilized cacao bark and cacao wood had a very distinctive fruiting habit, the macroconidia being borne on elongated sporodochia up to 10 mm. long and 1 mm. in diameter at the base tapering to a point. Measurements of representative spores are given in Table 1. Medium and age of culture had no appreciable effect on the dimensions

TABLE 1. *Spore measurements of* Calonectria rigidiuscula *in* μ

Ascospores:	3-septate, 27 (24–32) × 7 (6–9)
Conidia:	0-septate, 8 (6–11) × 4 (3–5)
	5-septate, 64 (52–73) × 7 (6–8)
	7-septate, 71 (65–81) × 8 (6–8)
	9-septate, 76 (71–88) × 8 (6–9)

of the spores in each class, but on the agar media 5- and 7-septate macrospores were most common, while on sterilized cacao wood and bark 9- and 10-septate spores predominated. None of the cultures produced perithecia.

CONDITIONS ASSOCIATED WITH *CALONECTRIA RIGIDIUSCULA* INFECTION

Acute dieback

Acute dieback is an extremely destructive disease, not previously described in the Gold Coast, which caused a considerable amount of damage during the 1944–5 dry season. A disease of a similar type, which was attributed to unsatisfactory soil moisture relations, has been recorded from Surinam (Fernandes & van Dyk, 1926). With acute dieback the damage within the outbreak area was usually very severe; large branches and often the crowns of the trees were killed, and occasionally dead trees were found. The leaves on the dead branches turned a dark brown and remained

attached for several weeks. A close examination of the dying branches showed that the dieback was spreading down the tree mainly in the cortex and cambium; the infected tissues were claret-coloured, and there was a clear line separating them from the healthy tissues. The infection usually started in wounds on the smaller branches, and spread in towards the centre of the tree, until the main trunk was reached; during this spread large branches and eventually the whole crown was cut off and killed by girdling. There was usually little spread down the main trunk, though in some cases this also was killed and the tree died. Infected trees formed watershoots freely on the healthy parts of the trunk. It was an unusual feature of the disease that, though not previously recorded in the Gold Coast, it appeared practically simultaneously in many small outbreaks scattered over a wide area.

A number of these outbreaks were examined; usually they were clearly defined and contained about thirty severely damaged trees surrounded by healthy-looking cacao, but in exceptionally dry districts large areas were sometimes killed either by disease or drought. There were sometimes signs of dieback on the sides of the healthy trees adjacent to those which were severely affected. Cultures were taken from the advancing edge of the dieback in all the outbreaks examined, the fungi most commonly recovered being *C. rigidiuscula* and *Botryodiplodia theobromae* Pat. Of the outbreaks examined, 23% yielded only the former, 32% only the latter, and 45% both fungi. Both fungi occur commonly on Gold Coast cacao, though neither of them is normally capable of causing the damage recorded here. The dieback followed an unusually dry year, and it seems probable that it must be attributed primarily to unsuitable environmental conditions.

The climate of the Gold Coast is similar to that of other cacao-growing countries. In the cacao belt the annual rainfall is in the neighbourhood of 60 in. distributed fairly evenly throughout the year. The atmosphere is usually saturated at night and, even in the dry season, the relative humidity seldom falls below 70% in the daytime; the atmospheric humidity, however, is subject to occasional violent fluctuations during December, January and February under the influence of the 'harmattan', a dry north-east wind blowing from the Sahara. While the harmattan is blowing, the average relative humidity falls to about 25%, a fall which is completed in 24–48 hr. (Chamney, 1928). Normally the cacao seems to suffer little as the result of the dry season, though the harmattan is naturally accompanied by some wilting of the leaves and young shoots, and it would seem probable that in normal years there is a sufficient reserve of moisture in the soil to supply the demands of the tree following the sudden reduction in atmospheric humidity. The year 1944, however, was abnormally dry, and the drought was especially marked in the last 6 months, when the rainfall was 37% below the average for the previous 20 years. The onset of acute dieback in drier areas immediately after the harmattan suggests that, when the air dried out, the trees were weakened to such an extent by the lack of moisture that they fell an easy prey to fungi which were already present, but which were held in check in normal conditions.

Chronic dieback

Chronic dieback has only recently attracted attention in the Gold Coast. There is every indication that the disease has been present for a long time but had previously been confused with the effect of chronic capsid attack, a condition which has been described by Voelcker & West (1940). It is widely distributed through the cacao area of the Gold Coast and also occurs in Nigeria. Dieback of a similar type has been recorded in most cacao-growing countries and has been attributed to *B. theobromae* (Nowell, 1921; de Jonge & Drost, 1909). Penetration of the wood has been noted as a characteristic of this disease in other parts of the world and is also a feature of the condition occurring in the Gold Coast. In this dieback *B. theobromae* is considered to be, at most, a weak parasite which is attacking a tree already weakened by unfavourable environmental conditions, insect attack, or disease. It has been suggested that *B. theobromae* is a pure saprophyte following invasion by another fungus (Briton-Jones, 1934).

In the Gold Coast well-established outbreaks of chronic dieback are very readily recognized; they are clearly defined and usually consist of between thirty and forty trees surrounded by healthy cacao. The trees themselves are reduced either to a single main stem or a main stem with only a few of the larger branches remaining; watershoots are formed freely on the living wood, but these are often sickly and chlorotic, and seldom develop far. Comparison between the girths of trees in diseased patches and the girths of the surrounding healthy trees suggests that, prior to attack, normal growth was made by the diseased trees. As in acute dieback, the infection spreads from the ends of the branches towards the main trunk, but in the chronic form the fungus spreads more rapidly in the wood than in the peripheral tissues. This gives a characteristic arrow-head pattern when the branch is split longitudinally (Pl. 2, fig. 4). In the peripheral tissues there is only a very thin zone which is claret-coloured and looks active. In the infected xylem three distinct zones can be distinguished: the long-dead wood which is blackish grey, a more recently infected claret-coloured zone which darkens on exposure, and finally a straw-coloured, water-soaked line separating the infected area from the healthy xylem. The extent of the claret zone varies considerably from specimen to specimen and probably depends on the activity of the fungus. The black and claret zones are always clearly defined, and the colour of the former seems to be due to contamination with *B. theobromae*. In the claret zone *C. rigidiuscula* occurs alone. The straw-coloured line rarely contains fungus.

Typical symptoms of chronic dieback have been found associated with trees that have been damaged severely, either by acute dieback, capsid attack, or by the falling of large forest trees. With fallen trees it is sometimes possible to date the initial damage accurately, and the dieback has been observed to destroy the greater part of the crown in as short a period as 6 months. The spread becomes very much slower when the main branches and trunk are reached.

Canker

Cacao canker, which results from infection by *Phytophthora palmivora*, has been described in some detail by Dade (1927). It is essentially a disease of the peripheral tissues and the fungus may be active for two or three years. During this period the cambium and the tissues outside it are killed. The damage occurs normally on old trunks and quite large areas may be involved. Eventually the development of the lesion is checked and the wound heals normally by the formation of callus at its edges; wood discoloration is confined to the part immediately adjacent to the diseased peripheral tissues. *P. palmivora* is completely eliminated from the healed cankers. In the Gold Coast marked discoloration of the xylem is characteristically associated with *C. rigidiuscula* infection and has also been noted following infection by the same fungus in Surinam (de Jonge, 1909). Superficially it is impossible to tell whether the canker is infected with *C. rigidiuscula* or not, since the behaviour of the peripheral tissues is essentially the same in either case, and the cankers appear to heal normally. The appearance and behaviour of the fungus in the wood is essentially similar to that already described for chronic dieback, and it is again characteristic that the damage in the wood extends farther than the damage in the peripheral tissues (Pl. 2, fig. 5). Cases have been recorded where the healing has been complete, and there was actually a layer of healthy wood occluding the infected zone in the xylem showing a condition parallel to that found in recovery from infected capsid attack. In individual cases the extent to which the wood was penetrated varied considerably, but the damage might extend into the centre of the trunk. In heavily cankered trees over half the cross-section of the xylem may show infection.

Capsid attack

The damage which has been attributed to capsid attack is one of the major problems facing the West African cacao industry, the losses caused having been estimated at 20% of the Gold Coast crop (Box, 1944).

There are two capsids of major importance which feed on the stems of cacao in the Gold Coast, *Sahlbergella singularis* and *Distantiella theobroma*. In natural conditions *S. singularis* shows a preference for mature trees, while *D. theobroma* prefers trees less than 6 years old (Cotterell, 1926); this difference is not, however, specific, and in artificial conditions both capsids feed readily on shoots from trees of all ages (Posnette, 1943). The early stages in the development of the lesions have been studied by Squire (in Anon. 1946): very soon after puncturing occurs, a water-soaked area appears on the stem and later becomes depressed through the collapse of the underlying tissues. Squire considered that the lesions were far larger than would have been expected simply as the result of mechanical damage, and attributed the greater part of the damage to a toxin injected by the insect at the time of feeding. After about 3 weeks these sunken lesions dried out and the bark began to split. Descriptions of the later stages of the damage are

confusing since the authors failed to realize the effect of *C. rigidiuscula* infection on the development of the lesions, and it is proposed here to consider in detail the development of the capsid lesions from the early stages.

During the present investigation studies on development have been confined to lesions on woody shoots. The lesions were made by one capsid caged for 24 hr. on the stem of a potted seedling 1–2 years old; accidental infection with *C. rigidiuscula* was avoided by using only capsids which had completed their final ecdysis in aseptic conditions, and by keeping the seedlings isolated from other cacao plants. As a further check, the presence or absence of the fungus in lesions of different types and ages was determined by culturing from typical specimens, thus accidental infections could be recognized readily. For the anatomical investigations hand sections of freshly collected material were cut, killed in a formalin-acetic acid-alcohol mixture and stained in safranin and Delafield's haematoxylin. The lesions made by the two species of capsid behaved in the same way, and no attempt has been made to distinguish between them.

The first effect, which followed soon after the feeding of the insect, was a collapse of the medullary ray parenchyma in the cortical region; this damage usually extended a short distance into the medullary rays in the xylem. The phloem, and the adjoining cambium were largely unaffected (Pl. 1, fig. 1). This damage has been attributed directly to a toxin injected by the insect and is the cause of the water-soaked area on the stem noted by other workers. Soon after attack, the plant formed a secondary wound cambium which cut off the dead cortical tissues, and reconstituted, *in situ*, the parts of the primary cambium which had been damaged in the medullary ray region. These changes were already well advanced after 12 days (Pl. 1, fig. 2). In the cortex most of the medullary ray tissue was cut off but very little of the phloem. When the primary cambium had been restored its behaviour was perfectly normal, and, after a comparatively short period, the only sign shown by the xylem that capsid damage had occurred was a line of disorganized cells occluded in the healthy tissue; this represented the damage caused in the xylem medullary rays by the toxin at the time of feeding. In the course of time the soft dead tissues fell away leaving a characteristic lesion on the stem, showing on its surface a fine network of dead phloem fibres (Pl. 1, fig. 4). A transverse section of a lesion of this type (Pl. 1, fig. 3) showed the ring of disorganized cells buried in healthy xylem tissue, but otherwise the development of the stem was normal. Occasionally there was a slight discoloration in the disorganized region of the xylem. The development of the lesions has not been followed beyond this stage, but there appears to be no reason why they should leave any permanent mark on the stem. (The effect of infection by *C. rigidiuscula* on capsid injury is considered on p. 53.)

Pathogenicity of *Calonectria rigidiuscula*

Inoculations of healthy cacao stems

Artificial inoculations were made with cultures of *C. rigidiuscula* from different sources into stems of all ages, from soft green shoots to trunks 15-20 years old, in various degrees of health, and at different times of the year. Successful inoculations were made only in wounded stems, but no special precautions to provide protection or to maintain humidity were required. The fungus was established without difficulty in stems of all ages. The inoculum was always taken from pure cultures, thus precluding the use of ascospores, which had not been produced in culture. The success of the inoculation was confirmed by culturing from the artificially induced lesion, and, since the colour of the strain recovered was the same as that inoculated, the colour variation of the fungus provided a useful check on the inoculation technique. There appeared to be no difference in pathogenicity between the strains used. The interval between inoculation and reisolation varied from about 6 weeks to about 8 months; reisolation was successful after the shorter periods, but there was little sign of damage to the tissues. After the longer periods distinctive lesions could be seen, but even these were small.

Inoculations of mature stems were made into small holes, about $\frac{1}{4}$ in. diameter, cut down to the cambium, from which the peripheral tissues had been removed. Either suspensions of conidia in water, or mycelium on agar were used as inocula. When the inoculations were examined after 6 months, there was no superficial difference between the inoculated wounds and the controls. However, if the inoculated bark was cut, the lesion in the peripheral tissues was larger than in the controls and there was a thin claret-coloured active zone from which the fungus was isolated. Healing was more advanced in the controls than in the inoculated wounds, but the presence of the fungus did not appear to do more than check the development of the callus tissue. The wood tissue beneath the callus seemed to develop normally; after about 6–8 months there was often a complete layer of healthy wood beneath the point of inoculation, separating the lesion in the wood from the lesion in the peripheral tissues. The lesions in the wood were considerably larger in the inoculated wounds than in the controls, and showed as black wet-looking patches from which black streaks extended for a few centimetres up and down the healthy wood in the stem. These streaks were occasionally seen in the controls, but they were smaller and less well marked. The vigour of the trees made no difference to the establishment of the fungus, but healing was more rapid on the more vigorous trees. In one set of inoculations, which were examined after 8 months, fewer than half the infected lesions were occluded, and the average length of the black streaks in the wood was 6·6 cm.; in the controls of the same age all the lesions were occluded and their average length was only 2·3 cm.

On the younger stems, ranging from $\frac{1}{2}$ to $1\frac{1}{2}$ in. in diameter, a different technique

was used: a patch of bark was raised, the conidia were placed beneath it, and the bark was bound back in place with raffia, giving in effect a bark graft. In the inoculated series none of these grafts remained alive as compared with about 44% in the controls. A fairly large patch of bark, measuring about $\frac{3}{8} \times 1\frac{1}{2}$ in., was damaged in these inoculations, and the appearance of the lesions was very similar to the canker lesions seen in the field. All the stems inoculated were growing vigorously, and in many cases the wood lesions had been occluded, and the healing completed after 7 months. The lesions in the wood were essentially the same as those described above in the older tissue.

On the soft green stems, spore suspensions in water were painted on the outside and wounds were made with a fine needle. Development was slow, but differences were visible after about 8 months, when the superficial tissues round the points of inoculation were dead, and in some cases small blisters were formed. The wounds in the controls were scarcely visible. There was also a marked discoloration in the xylem tissues which was absent from the controls. In sections it was sometimes possible to see fungal hyphae in the mucilage canals of the cortex.

All sets of inoculations demonstrated the ability of the fungus to establish itself in stem tissues of all ages irrespective of the condition of the trees. They also showed, however, that the fungus normally developed very slowly and was unable to compete in growth with the callus formed by healthy cacao, though it could remain alive occluded by healthy tissue. The numbers of inoculations made, and the degree of success achieved, are shown in Table 2.

TABLE 2. *Artificial inoculations with* Calonectria rigidiuscula

	Inoculated series		Control series	
Type of tissue inoculated	No. made	No. infected*	No. made	No. infected*
Old trunks:				
Wood	26	26	15	2
Bark	24	22	10	—
Young shoots:				
Mature	20	17	13	8
Green	15	12	6	—
Totals	85	77	44	10

* Determined by isolation from the lesions.

The development of infected capsid lesions

Since there was no satisfactory technique for producing infected capsid lesions in controlled conditions, the course of their development was followed by culturing from lesions of various ages in the field, and sectioning others which showed typical symptoms. The rate of natural infection was high (about 80% at Tafo, Gold Coast), so there was no shortage of suitable material. The sections of the infected lesions were prepared in the same way as the uninfected lesions except that the mycelium

in the tissues was stained with picro-aniline blue following a general staining of the ground tissues with safranin (Cartwright, 1929). Again only woody shoots, similar in age to those on which uninfected lesions were produced, were studied.

The presence of *C. rigidiuscula* did not affect the first stages of attack, but with infected lesions the damage was not confined to the medullary rays in the peripheral tissues, but spread into the xylem and phloem; after about 2 weeks a considerable area was involved (Pl. 1, fig. 5). The mycelium appeared to be confined to the unlignified regions, and was, in the main, intercellular in freshly invaded tissues, although occasionally haustoria with swollen tips were found within the cells; the development was especially strong in the phloem where the mycelium could be seen ramifying in tissues which showed no obvious signs of disintegration; this is illustrated in Pl. 1, fig. 6 where the mycelium is clearly visible in the parenchyma of the medullary rays, but is less obvious in the conducting tissue where it is strictly confined between the cells and shows only as an abnormal thickening and darkening of the cell walls. In the course of time the infected tissues die and the lesions heal by the development of callus at their edges; during this process they can be seen as deep-seated cankers on the stems (Pl. 1, figs. 7, 8). In infected lesions the damage to the xylem is severe, and they can be recognized by the discoloured areas on the xylem cylinder below the cambium (Pl. 2, fig. 3 B). With uninfected lesions these are either absent or very small. The final stage in the development of infected capsid damage can be recognized by the characteristic roughening of the bark (Pl. 2, fig. 1) which has previously been attributed simply to capsid attack (Mayné, 1917; Cotterell, 1926). The roughened bark appears as the lesions become occluded and probably persists through the whole life of the branch. It arises from a folded phellogen, but the irregularity in the phellogen is reflected neither in the cambium nor in the tissues which arise from it. The roughened bark is always associated with a series of black, distinct, oval lesions on the surface of a central cylinder in the xylem (Pl. 2, fig. 3), these show as a broken ring within the secondary xylem in transverse section (Pl. 2, fig. 2). The location of the lesions in the stem, and their similarity to the damage in the xylem found beneath young infected capsid lesions, strongly suggests that these occluded lesions are actually the site of the initial damage. *C. rigidiuscula* has been isolated regularly from these occluded lesions in stems of all ages, but has never been found either in the healthy wood occluding the lesions or in the peripheral tissues.

Detailed observations were also made on the damage sustained by woody stems following capsid attack. The uninfected lesions were strictly confined, and showed no sign of spreading after the initial damage was complete; from this it appeared that the direct action of the toxin injected by the insect was confined to the lesion and played no part in the death of the upper part of the shoot, except in so far as it caused damage to the vascular tissue and impeded the movement of nutrients in the stem; therefore a few lesions which ringed the stem were more likely to cause death than many lesions confined to one side of it. For this reason, each of the damaged

stems examined was placed in one of the four following classes according to the proportion of the stem which was ringed: I, up to $\frac{1}{2}$ ringed; II, $\frac{1}{2}$–$\frac{3}{4}$ ringed; III, $\frac{3}{4}$ to completely ringed; IV, completely ringed. Within these classes the stems were recorded as dead or alive. The stems with infected lesions were collected in the field, and those with uninfected lesions had been artificially infested with insects in the conditions described above. The data collected from these observations are presented in Table 3.

TABLE 3. *Damage due to capsid attack on woody stems*

	Infected lesions		Uninfected lesions	
Class	No. recorded	No. killed	No. recorded	No. killed
I	5	—	10	—
II	14	—	5	—
III	14	3	9	—
IV	24	22	8	—
Totals	57	25	32	—

During these observations none of the stems with uninfected lesions sustained any visible check to growth, though over half of them had suffered a heavy insect attack. On the other hand, nearly half the stems carrying lesions infected with *C. rigidiuscula* were killed. Stems which were less than $\frac{3}{4}$-ringed did not die, between $\frac{3}{4}$ and completely ringed the chances of recovery were fair, while the chance of survival for a completely ringed stem was remote. No data are available to show the effect of *C. rigidiuscula* infection on young shoots suffering from capsid damage, but the death of the shoot follows so soon after attack that it seems unlikely that a fungus would have time to develop.

Role of Calonectria rigidiuscula *in acute dieback, chronic dieback and canker*

The part played by *C. rigidiuscula* in the early stages of acute dieback appears to be that of a mild parasite in a weakened tree. All the evidence collected during these investigations indicates that in normal trees the development of the fungus in the peripheral tissues is checked relatively easily; if this is the case *C. rigidiuscula* only assumes primary importance after the acute phase of the disease is passed, since trees infected with other mild parasites might be expected to make some recovery when conditions improve, while those infected with *C. rigidiuscula* continue to die back, though more slowly, owing to the continued spread of the fungus in the healthy wood.

In canker infection it is probably the presence of the fungus in the xylem which can do most serious harm to the tree by interfering with the supply of water and nutrients. On a vigorous tree one or two infected cankers probably do little harm, but the cumulative effect of many infected cankers, each occluding a sector of the xylem, may well prove serious, and it should also be noted that as the tree is progressively weakened the fungus will spread more rapidly, and the rate of decline will

be accelerated. It has been noted in the field that trees suffering severely from canker infection also frequently show signs of dieback, but in this case it is difficult to separate cause from effect since it can be argued either that the canker infection has rendered the tree susceptible to dieback, or that the presence of dieback has supplied a source of infection for the cankers.

Chronic dieback in most cases appears to be the end stage of *C. rigidiuscula* infection rather than a disease in its own right. *C. rigidiuscula* seems to spread readily in trees which have been weakened by a variety of causes, and if the vigour of the tree has been reduced by infected capsid attack, acute dieback, or infected canker, not only is it in a suitable state for the development of the fungus, but the fungus is already present and active, so that chronic dieback is almost inevitable. The source of trouble is again the slow but continued spread of the fungus in the xylem which precludes the recovery of the infected trees.

Discussion

The results of the inoculation experiments demonstrated that *C. rigidiuscula* could be established without difficulty in healthy stems; the fungus must therefore be considered a wound parasite of cacao. In healthy cacao the development of the lesion was slow. In the peripheral tissues the fungus seemed unable to compete with the developing callus tissues and was only able to retard the healing of the wound; to judge from the behaviour of infected capsid lesions, the infection is eventually eliminated completely from the peripheral tissues. In the xylem the fungus appeared to be rather more successful; the development was more rapid and extensive and the fungus could remain alive for long periods. But even here it seemed that the spread was checked, since the lesions occluded in the xylem following infected capsid damage appeared to be approximately the same size as those found below the peripheral tissues when the damage was fairly recent, even though in the former the infection might have been present for 10 years or more. From this it would appear that a normal vigorous tree is unlikely to suffer severely when infected by *C. rigidiuscula*, and the chief source of danger lies in the effect of fungal attack on a weakened tree. It seems probable that old trees will succumb more readily to *C. rigidiuscula* infection than young, and the presence of the fungus may well be one of the more important factors which limits the life of West African cacao. On vigorous trees the importance of the fungus appears to lie in its ability to intensify the effect of mechanical injury. This is particularly illustrated by its relation to capsid attack.

Before it is possible to evaluate the damage caused to cacao by the infection of capsid lesions by *C. rigidiuscula*, some estimate must be made of the damage caused by the capsid alone. The capsid can kill only young green shoots, so its damaging effect will be determined by the ability of the cacao to bear the loss of these shoots without suffering permanent harm. In normally growing cacao, periods of rapid stem elongation, referred to as 'flush', alternate with periods when no elongation

occurs, and it is only during the periods of elongation and for a short time after them, when the tissues of the stem are maturing, that the cacao can suffer severe damage from capsid attack. Even in these periods only partial defoliation can occur, since a flushing tree normally carries the leaves of the two preceding flushes; furthermore, the damaged shoots will usually be replaced in a short time by the development of lateral buds. In these circumstances it would appear that only a continuous, heavy capsid infestation could induce dieback; in practice this seldom occurs.

Capsid damage which is infected with *C. rigidiuscula*, however, presents an entirely different picture, since large shoots, at least up to 1 in. in diameter, can be killed, with the result that a heavy infestation, when the lesions are infected, can produce a state approaching complete defoliation at any time in the year. This defoliation does not represent the sum total of the damage which may be expected, since it is likely to weaken the tree sufficiently to allow the spread of dieback, and each of the defoliated shoots is a potential point of infection from which the dieback may spread. This relationship between capsids and dieback may explain the presence of pockets of chronic damage in areas infested with capsids and also their similarity to patches of chronic dieback. It appears that the true position with regard to capsid attack is that neither the insect nor the fungus alone normally does serious damage to the tree, but in combination their damage is one of the major problems facing the cacao industry in West Africa.

The effect of *C. rigidiuscula* infection on canker is rather more difficult to elucidate since *P. palmivora* by itself can cause damage to the cacao plant, though this damage may not be serious if there are only a few cankers. *P. palmivora* develops in the peripheral tissues, and the infection is completely eliminated when the diseased tissues have been thrown off; there is little damage to the xylem. When *C. rigidiuscula* infection is present an appreciable portion of the xylem may be diseased, and this may well weaken the tree by disturbing the flow of nutrients; further, the infection is not eliminated when the canker heals, but remains as a potential source of danger.

Acute dieback probably does not represent a serious menace to the cacao industry. In areas that are affected the disease may do serious harm to the trees, but it seems likely that in these areas environmental conditions are unsuited to cacao cultivation. In borderline areas, where the pockets of infection are scattered, removal of sources of infection may reduce the intensity of the disease, but it must be remembered that, in the absence of *C. rigidiuscula*, other fungi may attack drought-weakened trees. There is no reason to suppose that this condition will occur in the good cacao areas.

Chronic dieback cannot strictly be considered as a disease, but is rather the chronic final stage of conditions which weaken the tree such as drought, infected capsid attack, or serious mechanical damage. Infected trees are unlikely to recover, and recognition of the disease permits the removal of unthrifty trees.

Notes on control

The present knowledge of *C. rigidiuscula* infection is not sufficient to allow detailed recommendations to be made for the control of the fungus, but it does open up possible lines of approach. The first is obviously to try to reduce the reservoir of infection in the field; since *C. rigidiuscula* sporulates most readily on dead wood, the most important pockets of infection are likely to be chronic dieback patches, and these should be eliminated, either by coppicing the trees or by removing them entirely. This treatment would be worth while in any event since it entails the replacement of unproductive trees by trees which will eventually bear fruit. To make this method of control effective all the dead wood should be removed from the farm. This should not prove a serious economic burden to the farmer, since dead cacao has a certain value as firewood. Once the trees have been infected, spraying is unlikely to have any effect on the fungus, but the possibility of protective spraying to reduce the infection of capsid lesions should be considered, particularly on young trees which are readily accessible. A great deal more knowledge of the fluctuations in capsid population and the methods of fungal infection in the field will be required before a spraying programme can be worked out, but, if the plants can be covered with a persistent fungicidal spray to kill the free spores immediately before infestation of capsids becomes heavy, a considerable reduction in the incidence of infected lesions might result, with a consequent reduction in the total damage. Control of the fungus may provide an alternative to control of the capsid in reducing damage should the latter prove impracticable.

The author wishes to express his gratitude to Mr F. A. Squire for his suggestions in the early part of the work, and for the use of plants which he had infested artificially with capsids; to Mr D. J. Billes for carrying out the later artificial infestations; to Mr J. McA. Todd for the records of *C. rigidiuscula* in Nigeria, and to Mr J. Nicol and Mr R. J. Benstead for the photography: also to Dr S. P. Wiltshire of the Imperial Mycological Institute for his advice during the course of the work, to Mr E. W. Mason of the Imperial Mycological Institute for his assistance in identifying the pathogen, and finally to the Director of Research, West African Cacao Research Institute for permission to publish this paper.

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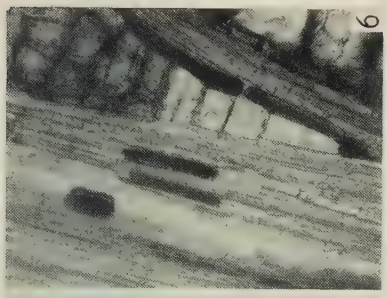
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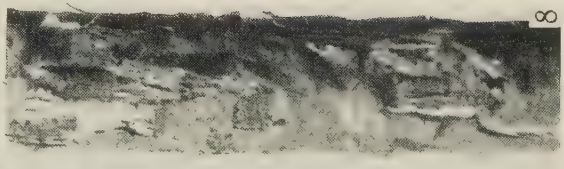
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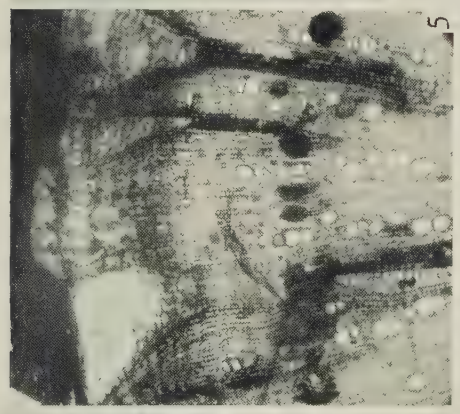
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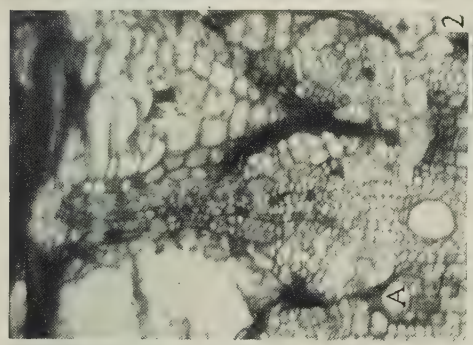
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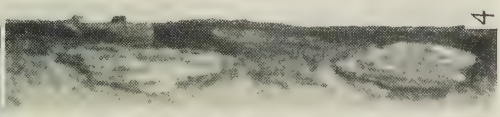
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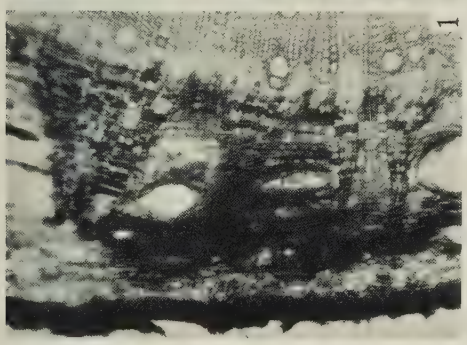
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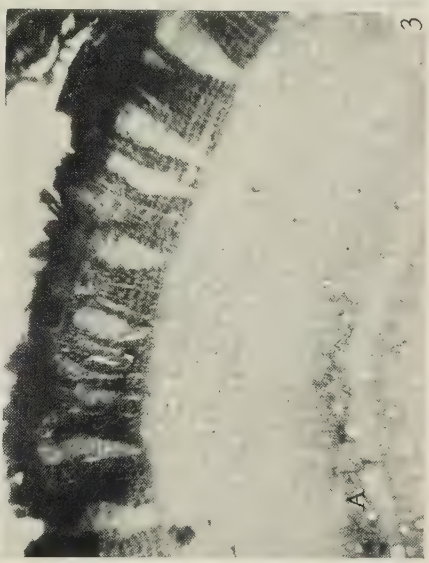
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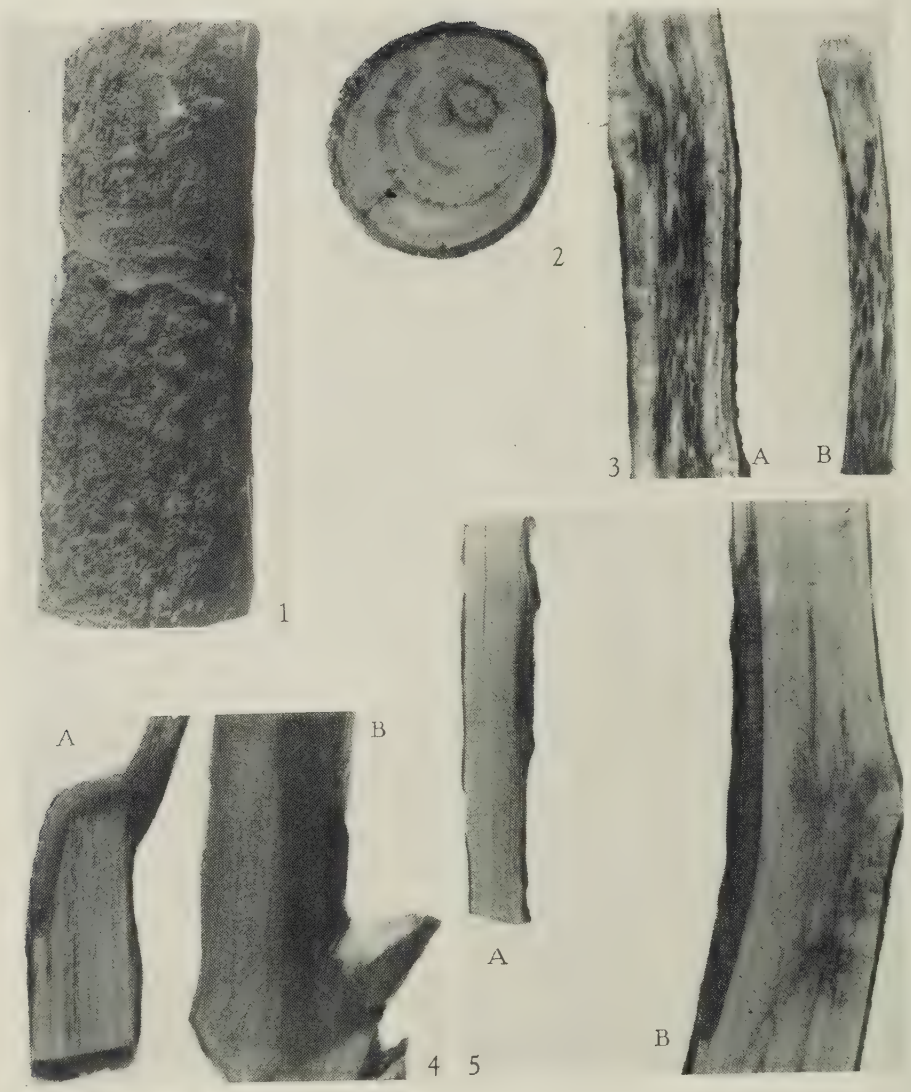

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EXPLANATION OF PLATES

Plate 1. Development of capsid lesions

Fig. 1. Transverse section uninfected lesion 3–4 days after attack (× 40).
Fig. 2. Transverse section uninfected lesion 11–12 days after attack (× 75). A, damage to medullary rays in the xylem.
Fig. 3. Transverse section uninfected lesion 9 months after attack (× 20). A, damage corresponding to A in fig. 2.
Fig. 4. Uninfected lesion 9 months after attack, surface view (× ½).
Fig. 5. Transverse section infected lesion 2–3 weeks after attack (× 25).
Fig. 6. Longitudinal section phloem of lesion in fig. 5 showing mycelium (× 100).
Fig. 7. Transverse section infected lesion 9 months after attack (× 10).
Fig. 8. Infected lesion 9 months after attack, surface view (nat. size).

Plate 2

Fig. 1. Roughened bark associated with past infected capsid attack (× ½).
Fig. 2. Transverse section stem with roughened bark showing infected lesions in xylem (× ½).
Fig. 3. Damage to xylem associated with infected capsid attack (× ½). A, old damage dissected to show lesions on central cylinder in xylem; B, lesions on xylem associated with recent damage.
Fig. 4. Chronic dieback (× ⅓). A, broken stem uninfected; B, broken stem infected with *C. rigidiuscula*.
Fig. 5. Canker (× ⅓). A, without *C. rigidiuscula* infection; B, infected with *C. rigidiuscula*.

NOTES ON A VIRUS DISEASE OF CACAO

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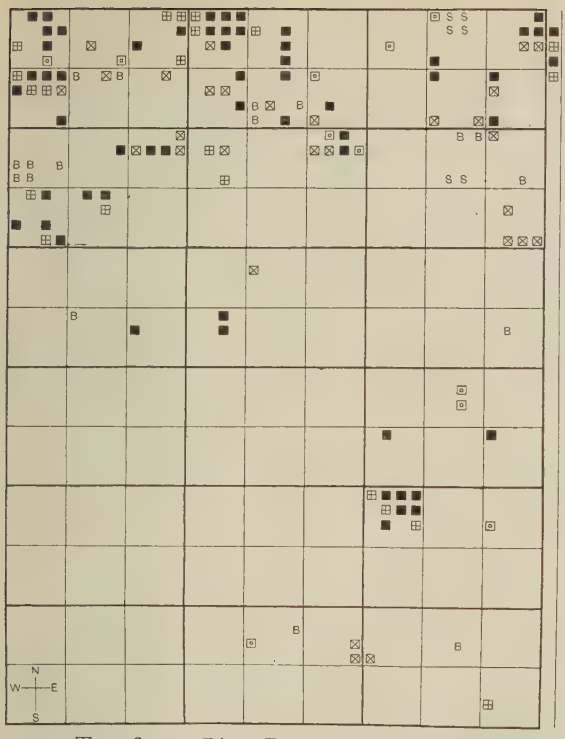
(With Plate 3 and 6 Text-figures)

A virus disease of cacao, discovered in Trinidad towards the end of 1943, is apparently confined to the north-western corner of the island and does not occur in the main cacao-growing areas of eastern, central and southern Trinidad; nor has it been found in Venezuela, Tobago or the Windward Islands of Grenada, St Vincent, St Lucia and Dominica. The symptoms resemble those of West African swollen shoot disease, consisting of vein-clearing or mosaic of the leaves and red-mottle of the leaves and pods. No swellings are formed, but recent observations indicate that the disease may cause both dieback and reduction in yield. There are two strains closely resembling each other. The spread of the disease under field conditions has been demonstrated, and although the rate of spread has varied somewhat, a mean increase of 41 % over the original number of infected trees has been found over a 10-month period. As with swollen shoot disease, existing diseased areas have increased in size and new outbreaks at short distances have occurred. Both strains were readily transmitted by budding, the incubation period varying from 34 to 136 days, with a mean around 90 days, but no insect vectors have been found. There is at present little reason to believe that any varieties of cacao are immune to the disease or are even symptomless carriers. The question of eradicating this virus, while its distribution remains limited, should be seriously considered.

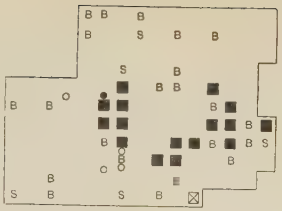
Introduction

Posnette (1944) described a disease of cacao from Trinidad which he attributed to two viruses or virus strains, for which the names 'red-mottle' and 'vein-clearing' were suggested. Experiments involving the transmission of these by budding showed that although reactions varied on different types of cacao their symptoms remained distinguishable. Further work has shown that although these strains are undoubtedly distinct, they are very similar, and that certain of Posnette's tentative conclusions require modification.

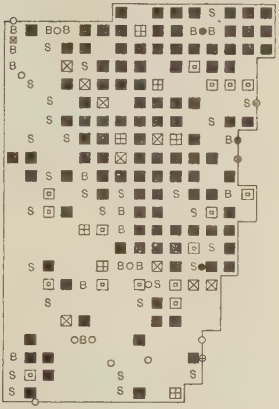
A field survey, carried out by the Trinidad Department of Agriculture, has shown that the disease is confined to the western end of Trinidad's Northern Range, and in that area mainly to the valleys of Diego Martin, Santa Cruz and Maracas, where large cacao estates are situated (Text-fig. 1). It is likely that the incidence of the disease is greatest in the Santa Cruz valley, which lies between Diego Martin and Maracas, and both strains are found there. Apparently strain A alone is found in Diego Martin and strain B alone in the Maracas valley. The disease does not occur at present in the main cacao areas of eastern, central and southern Trinidad; nor is it known from neighbouring cacao-growing countries. Posnette & Palma (1944) failed to find the virus in eastern Venezuela, and Dale found no trace of it in Grenada, St Vincent, St Lucia or Dominica on recent visits. So far, there has been no report of the disease from Tobago.



Text-fig. 2. River Estate, Diego Martin.

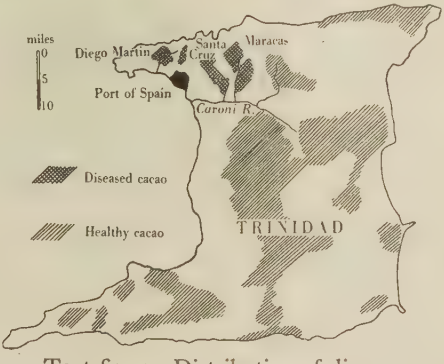


Text-fig. 4. Isolated diseased area, Santa Cruz valley.

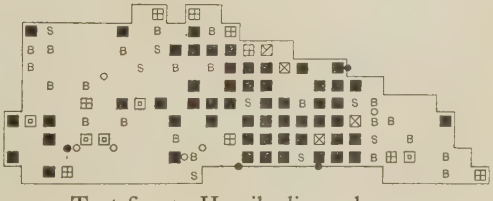


Text-fig. 6. Heavily diseased area, Santa Cruz valley.

Text-fig. 3. Isolated diseased area, Maracas valley.



Text-fig. 1. Distribution of disease.



Text-fig. 5. Heavily diseased area, Santa Cruz valley.

■ Tree diseased in April 1944.
⊡ Tree diseased in July 1944.
⊞ Tree diseased in October 1944.
⊠ Tree diseased in February 1945.
○ Tree out of place.
B Blank. S Small tree. All trees planted at 12 × 12 ft.

The symptoms show some resemblance to those of West African swollen shoot disease, but, so far as is known at present, no swellings like those associated with certain strains of that virus are formed. Leaf symptoms consist of vein-clearing, mosaic and red-mottling, and in certain yellow-podded strains of cacao a red-mottle is found on the young pods as well. Posnette was of the opinion that the affected trees often showed signs of dieback and poor health, but owing to the general bad condition of the cacao in areas where the disease occurs, definite proof could not be obtained. More recently, observations on a field of 8-year-old vegetatively propagated I.C.S. clones, in which the disease is spreading, have indicated that it can cause both dieback and loss of crop. The two strains, described by Posnette as 'red-mottle' and 'vein-clearing', will be known in this paper as 'strain A' and 'strain B' respectively. Posnette's names are untenable, since on certain types of cacao the symptoms of his 'vein-clearing' strain include red-mottling of the leaves, and on others the 'red-mottle' strain produces mosaic symptoms only.

Symptoms

Strain A

On many cacao varieties this strain, common in the Diego Martin and Santa Cruz valleys and designated by Posnette as 'red-mottle', causes a red pigmentation of the tissues bordering some or all of the main veins of the leaf (Pl. 3 *a*). Young cacao leaves vary in colour, in different varieties, from pale green through pink to dark red, and this symptom is not visible on trees whose young leaves are dark red, but is clearly defined on those which 'flush' green or pink. As the leaves harden and turn green the red pigmentation fades and disappears. Sometimes the red-mottle covers the whole leaf, but more frequently only a few veins are picked out with the reddish feather-like pattern. Some form of mosaic usually accompanies the red-mottle, but is notably absent on I.C.S. 6, 53 and 60, with the result that unless young leaves are present the disease cannot be diagnosed on these clones. The mosaic usually takes the form of cleared areas, varying in size and shape, bordering the main veins of the leaf. There is no definite 'vein-clearing', although on some clones, such as I.C.S. 98, this condition is approached. Mosaic symptoms differ markedly from tree to tree in seedling plantations and from clone to clone in plantings of vegetatively propagated trees. Thus, the clones I.C.S. 3 and 7 show very well-marked mosaic symptoms (Pl. 3 *b*), while the mosaic produced on I.C.S. 1 is very slight and often difficult to recognize. Mosaic is usually associated with red-mottle, but the latter is not found on certain varieties. Cacao trees in Trinidad put out new leaves four or five times a year in definite 'flushes' of growth; not all the leaves of any one flush will show the symptoms described above. It has often been observed that the lowest or first produced leaf of the new growth on a shoot is not only the likeliest to show symptoms, but will do so more markedly than subsequently produced leaves, which are often symptomless. Strain A also causes red blotches on the young pods of

certain yellow-podded cacaos, e.g. I.C.S. 6 and 8. According to Posnette, crinkling of the leaf and necrosis of the leaf-tip may be associated with this strain, but we have found these neither constant nor reliable symptoms for diagnosis. Recent observations indicate that this strain can cause dieback and reduction in yield in comparatively young trees.

Strain B

The second strain, described by Posnette as 'vein-clearing', occurs in the Maracas valley and parts of the Santa Cruz valley. Although this strain is very similar to strain A, inoculation experiments have shown that slight though constant differences exist. The chief difference is that on most types of cacao a pronounced vein-clearing, often extending to the fine veins of the leaf, occurs, and this persists when the leaves harden (Pl. 3 *d*, *e*, *f*). The clone I.C.S. 6, when infected with strain A, develops red-mottling only; but mosaic as well as red-mottling is produced on its leaves by strain B (Pl. 3 *c*), the result being indistinguishable from the symptoms of strain A on certain other clones. I.C.S. 6 can therefore be used as a differential host for identifying the two strains. According to Posnette, leaf crinkling is more pronounced with this strain than with strain A, but in our opinion this symptom is again neither constant nor reliable. The effect of this strain on the condition and yield of cacao has not yet been assessed, due to the general bad condition of almost all trees, infected or otherwise, in the areas where it occurs. When double inoculations, using buds infected by different strains, were made on the same date, the symptoms subsequently appearing on the stocks were those of strain B.

Field observations

Experiments were started early in 1944 to determine the rate of spread of the disease in the field. For this purpose the field of I.C.S. clones, planted at River Estate in the Diego Martin valley as a series of randomized block experiments, was available (Text-fig. 2), and its study has proved of the greatest value and interest. In addition, isolated patches of diseased cacao in the Maracas and Santa Cruz valleys (Text-figs. 3, 4) and two heavily diseased patches, also in the last-named locality (Text-figs. 5, 6), were mapped. Each of these patches was surveyed at approximately 3-monthly intervals with the results shown in Text-figs. 2–6 and Table 1. It will be noted that

Table 1. *Increase of cacao virus during* 10 *months ending* 28 *February* 1945

			No. infected					
		No. of trees	Apr. 1944	July 1944	Oct. 1944	Feb. 1945	10 months increase	10 months increase %
Diego Martin	1	592	47	54	71	96	49	104
	2	576	5	7	7	8	3	60
	3	576	6	8	12	15	9	150
Santa Cruz	1	173	58	63	72	76	18	31
	2	127	17	17	17	18	1	6
	3	281	113	131	139	148	35	31
Maracas	1	438	75	75	81	94	19	25
Totals		2763	321	355	399	455	134	41

the numbers of diseased trees have increased in every case, but that the rate of spread has varied somewhat, with a mean increase of 41% over those originally infected during a 10-month period. Existing groups of diseased trees have expanded, the larger more rapidly than the smaller ones, and new outbreaks at a short distance have occurred. This type of spread is in agreement with West African experience with swollen shoot (Posnette, 1943).

The field of I.C.S. clones at River Estate (Text-fig. 2) is especially suitable for studying the effect of the disease on the condition and yield of the trees, as the oldest trees in the field are now 8 years old and are approaching full bearing, and individual tree yields are kept. No significant difference could be detected between the yields of healthy and diseased trees for the year 1943, but by September 1945 the poor condition of some of the originally diseased trees had become so marked that more detailed observations were made. Counts were made of the number of trees

Table 2. *The effect of virus infection on condition of trees in field 7, River Estate, Diego Martin*

	Virus-free	Virus-infected (recent)	Virus-infected (original)	Total
Trees free of dieback	390	43	23	456
Trees with dieback	56	20	22	98
Total	446	63	45	554

Table 3. *Yield in pods, August 1944 to July 1945 (inclusive) for set 1, field 7, River Estate, Diego Martin*

	Virus-free	Virus-infected (recent)	Virus-infected (original)
Total pods	7407	1086	566
No. of trees	446	62	46
Pods per tree	16·6	17·5	12·3

showing dieback symptoms in populations of virus-free and virus-infected trees, the latter being divided into two groups consisting of trees infected before and after February 1944 (see Table 2). The application of the Exact Treatment for 2×2 Contingency Tables (Fisher, 1942) leads to the following conclusions:

(1) There is a highly significant difference between the proportion of trees suffering from dieback in the virus-free and virus-infected populations (i.e. the virus has significantly increased the number of trees which are deteriorating).

(2) There is a similar highly significant difference between virus-free trees and trees infected before February 1944, and also between virus-free trees and trees infected after February 1944.

(3) There is no significant difference in this respect between trees infected before and after February 1944.

In addition, the yields for the year August 1944 to July 1945 (inclusive) were examined (see Table 3). The following facts were demonstrated:

BAKER and DALE—*Notes on a virus disease of cacao*

(1) There is a highly significant difference between the yields of healthy trees and trees diseased in February 1944.

(2) There are indications of a difference (probability just over the 5% point) between recently diseased trees and trees infected before February 1944.

(3) There is no significant difference between the yields of healthy and recently diseased trees, or between the yields of healthy trees and all the diseased trees together.

These results are taken as an indication that the disease can cause both dieback and loss of crop.

Transmission

Posnette readily transmitted both strains by budding. With strain A the interval between budding and the appearance of symptoms varied from 94 to 119 days, while with strain B two of his plants developed symptoms in 45 and 60 days respectively, suggesting a shorter incubation period for this strain. Our results indicate that this difference was probably due to chance, and that the strains cannot be differentiated in this way. The maximum and minimum incubation periods found in our series of experiments were: for strain A, 125 and 34 days; for strain B, 136 and 61 days, respectively. No symptoms of either strain can be observed until the plants flush, and if this occurs shortly after budding and the next flush is long delayed, a very long 'incubation period' will inevitably result. The mean of 90 days obtained for the incubation period should only be taken as a rough guide, as this period will be shortened or greatly increased by favourable or unfavourable conditions for the growth of cacao. The length of the incubation period and the frequent rarity of symptoms even on an artificially inoculated tree, especially if infection is recent, suggests that this virus moves very slowly through the host tissues.

No vectors have so far been discovered, but two or more mealy bugs, probably including *Pseudococcus citri* Risso and *Ferrisiana virgata* Ckll., and an aphid, *Toxoptera aurantii* Boyer, occur fairly commonly in Trinidad cacao. West African experience indicates that one or more of them may transmit the disease.

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EXPLANATION OF PLATE 3

a, Red-mottle alone, as produced by strain A on I.C.S. 6. The white flecks are due to holes in the leaf, not mosaic. *b*, severe mosaic, as produced by strain A on I.C.S. 3. *c*, red-mottle and mosaic as produced by strain B on I.C.S. 6. *d*, vein-clearing, strain B; extending to the smaller veins. *e*, vein-clearing, strain B; severe symptoms; note necrosis of the leaf-tip. *f*, vein-clearing, strain B; especially near the larger veins.

WIREWORM POPULATIONS IN RELATION TO CROP PRODUCTION

II. POPULATION CHANGES IN GRASSLAND

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(With 3 Text-figures)

Six grass fields were sampled once a fortnight from July 1943 to September 1944, and the wireworms in the samples counted by the washing and flotation method.

The counts from twenty standard samples showed huge fluctuations which rendered them practically valueless as estimates of wireworm populations when treated singly. When treated as running means of four consecutive samplings, the counts showed certain seasonal trends, with minimum populations in July and August, rising populations throughout the autumn, maximum populations in the months from January to April and a sharp decline to the minimum populations again from April to July. On the average, the counts taken in winter were twice as high as those taken in the summer months.

The fluctuation was evident in all size groups based on larval length but was most marked in the wireworms 'under 4 mm.', '4 mm.' and '5 mm.'

Samples taken to depths of 12 and 24 in. showed that on the average about 75% of the wireworms were found in the 0–6 in. layer and about 90% in the 0–12 in. layer. These proportions varied considerably with the seasons.

The seasonal changes in wireworm populations observed do not correspond with those expected from the life history of *Agriotes* spp. Possible explanations for this anomaly are discussed, but it cannot be attributed to any known technical or biological factor. Several possibilities remain to be explored.

Introduction

Critical studies of wireworm populations have only recently become possible with the development by Salt & Hollick (1944) of an accurate method for separating wireworms from soil. A modified form of this method designed, in the first instance, for rapid field survey (Cockbill *et al.* 1945) appeared to give results of sufficient accuracy to recommend its use for investigational work. In this and subsequent papers we shall describe the application of this method in studies on population changes under different conditions in agricultural land.

During 1943 and 1944 in the Eastern Counties Advisory Province, an investigation was begun to record the effects of cultivations on wireworm populations in land newly broken from grass. Six permanent pastures were selected which were to be ploughed during the summer or autumn of 1943 for spring cropping. Sampling was carried out on these fields at fortnightly intervals before sowing. As a control, the wireworm populations of unbroken permanent pasture were recorded by taking

samples at similar intervals from six grass fields. It soon became evident from the results that the wireworm populations of the grass fields were showing unexpected numerical and structural changes. It became necessary, therefore, to treat this part of the work as a separate investigation. Subsequent papers will deal with the concurrent investigations on the effects of cultivations.

Methods

(*a*) *Sampling.* The sampling technique adopted in these investigations is essentially that described for the wartime Wireworm Survey in official reports (*Bull. Minist. Agric.* no. 128). Twenty samples were taken at fortnightly intervals from points distributed more or less evenly over the fields. Although the technique of using random numbers was not adopted in taking the samples, they can be regarded as random for most purposes. Each sample was taken with a cylindrical borer of 4 in. internal diameter (1 : 500,000 of an acre). In three fields the samples were taken to a depth of 12 in., the soil from the 0–6 and 6–12 in. layers being kept separate. In the other three fields, samples were also taken from the layers 12–18 and 18–24 in., so that information would be available about the movements of wireworms to and from the deeper soil layers. The samples were bulked in lots of ten and transferred in the field to 4 gal. tins.

(*b*) *Extraction of wireworms from samples.* The technique used to separate the wireworms from the soil has been fully described by Cockbill *et al.* (1945). The samples were soaked in water in the tins until the soil structure had partly broken down, a process requiring 2–5 days. They were then washed by means of a strong jet of water through a bank of sieves of meshes 2, 5 and 60 to the inch. The coarser debris and stones were retained on the 2- and 5-mesh sieves, and the wireworms were collected with the finer debris and silt on the 60-mesh sieve. The latter with its contents was immersed in a tank of brine of specific gravity 1·15–1·20 and the floating material skimmed off into a collecting sieve (60-mesh). After boiling to expel air from the plant debris to enable it to sink, the wireworms were collected in a layer of paraffin over water. Lengths were measured to the nearest millimetre and the totals in each size group recorded. Only *Agriotes* spp. were included in these records. Other species were recorded separately, but their numbers were small compared to the numbers of *Agriotes* spp. collected.

The efficiency of the apparatus has been the subject of numerous tests as described in the preceding paper. These tests showed that an average of 95% of all wireworms was extracted from the samples. Actual efficiency, however, depended on the care taken with each operation during the extraction. Residue tests, in which the material remaining after each operation was re-examined, were instituted as a routine carried out at regular intervals. During the investigations herein described the residue tests gave us no reason to believe that the extraction varied from the average efficiency of 95% originally claimed. This question will be considered further in the discussion.

Results

Three adjacent pairs of permanent grass fields in Cambridgeshire and Huntingdonshire were selected for this investigation.* The only factors considered in selecting the fields were ease of access and absence of stones which would make sampling difficult. The soils on which the fields were situated were classified as follows: fields I and II on silt overlying Boulder Clay; fields III and VI on Gault Clay; fields IV and V on medium loam overlying Boulder Clay. All fields had been in grass indefinitely except field VI which had been ploughed and directly reseeded some 10 years previously. All had been grazed and hayed and could be described as medium-quality pastures.

The total wireworms recovered from each field are shown in Fig. 1, which shows the actual numbers of wireworms recorded at each sampling. In addition, the total population, the populations of wireworms '4 mm. and over' and 'under 4 mm.' in length are expressed as running means of four consecutive samplings. This running mean is obtained by taking the means of the 1st, 2nd, 3rd and 4th samplings; of the 2nd, 3rd, 4th and 5th; and so on. Its use has been discussed by Williams (1940). Its value here lies in the fact that the curves so obtained show greater uniformity between fields than do the untreated diagrams, and by smoothing out the variation between successive samplings it shows up the trends in populations. There are good grounds for believing that at least 100 samples are required in order to obtain estimates of the population in which the sampling error is negligible (private communication from Mr D. J. Finney). It was not possible to sample these fields so intensively, but each point on the curves of the running means represents the mean population of the field as obtained from eighty samples taken over a period of 8 weeks.

Changes in the populations

(*a*) *Variation between samplings.* It is evident from Fig. 1 that the variation in the fortnightly population estimates in all fields throughout the investigation was sufficiently great to render any single sampling result based on twenty samples of little value as an estimate of the population when considered alone. Thus, in field VI, where sampling was maintained to a depth of 24 in. and, therefore, where the most complete population estimates should have been recorded, the first sampling in August 1943 yielded 182 wireworms, the third sampling in March 1944, 350 wireworms and the first sampling in August 1944, 133 wireworms. Other population estimates on this field fell anywhere between these limits, but no single estimate could be said to indicate the 'true' population of the field.

It appears from the foregoing results that the improvement of the extraction technique has not reduced the amount of fluctuation in successive estimates. It is

* Field I: Brampton 44 (Hunts)
Field II: Brampton 47 (Hunts)
Field III: Girton 166 (Cambs)
Field IV: Godmanchester 49 (Hunts)
Field V: Godmanchester 72 (Hunts)
Field VI: Madingley 3 pt. (Cambs) (Spinney Pasture)

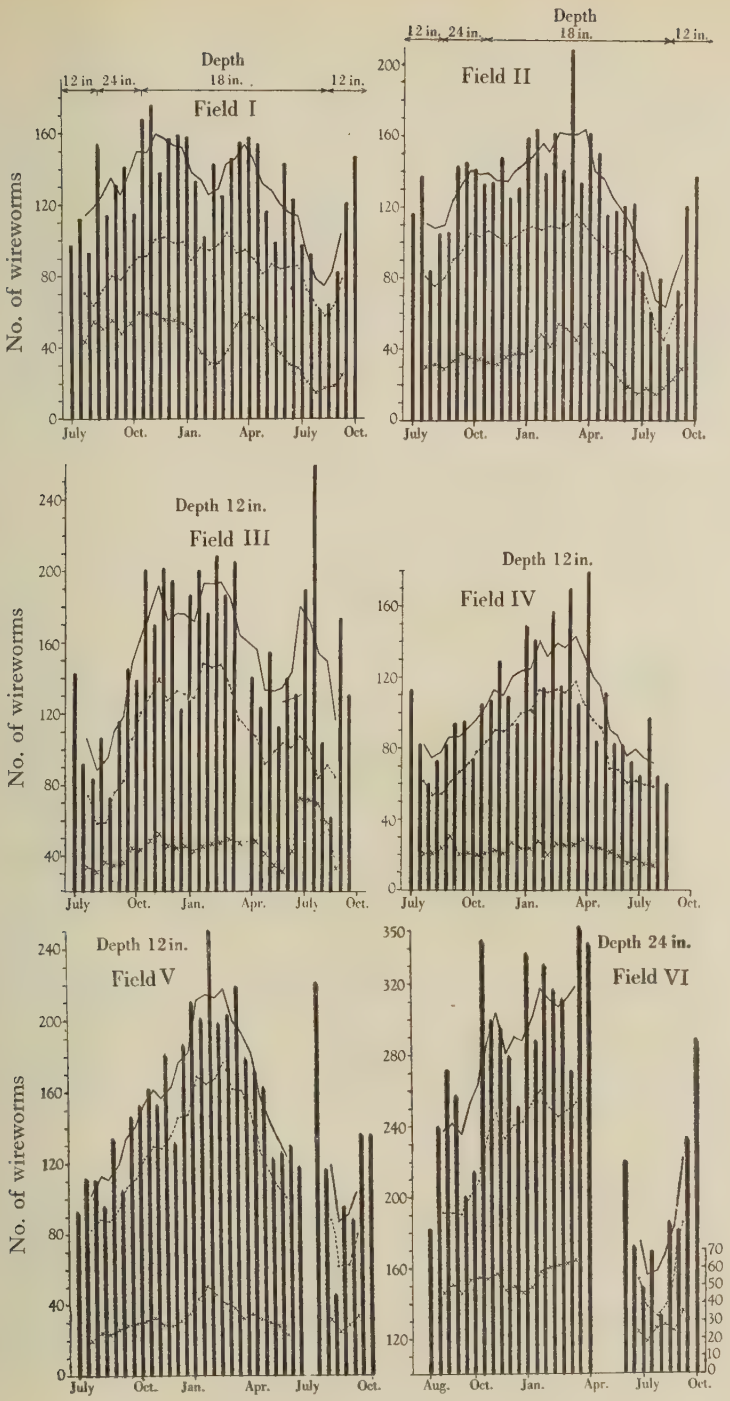

Fig. 1. Fields I–VI. Wireworm numbers at successive samplings.

| Total wireworms. ——— Running mean of total wireworms.
-·-·- Running mean of 4 mm. and over. -×-×- Running mean of under 4 mm.

clear that many more than twenty samples are required if estimates are not to fluctuate between very wide limits, even with improved laboratory methods. In precise work, this factor of sampling error outweighs in importance the extraction of every wireworm in the samples.

(*b*) *Seasonal fluctuations.* The most noteworthy feature of the population estimates in Fig. 1 is the pronounced seasonal fluctuation that appears. In all six fields the estimates showed a minimum in July and August 1943, a maximum in January to April 1944, and a minimum again in July and August 1944. This seasonal fluctuation was evident in the wireworms 'under 4 mm.' and '4 mm. and over' as seen from the respective running means.

The figure shows clearly the magnitude of this apparent increase in population (more than twofold in field VI) and the extraordinary consistency with which it occurred in the different fields. It needs no further description and will be discussed fully later.

(*c*) *Changes within the size groups.* All wireworms collected in the investigation were measured to the nearest millimetre and recorded in groups of 1 mm. interval from 4 to 25 mm. Wireworms of length less than 4 mm. were grouped together as 'under 4 mm.' and included wireworms as small as 1·5 mm. It was decided to group these smallest forms together, since the apparatus had not been carefully tested at the time in the recovery of wireworms 'under 4 mm.' and therefore a fine subdivision of these small larvae seemed unjustified. Furthermore, as we were separating the larvae only to the nearest millimetre, the error in measuring the smallest forms would be so great that any figures obtained would only be rough approximations. Nevertheless, the numbers of wireworms 'under 4 mm.' proved to be quite large, and as their behaviour is an important factor in the changes in the total population, we have treated them as valid estimates.

The actual numbers of wireworms recovered are shown arranged in size groups in Fig. 2 for field I only, the others being similar. Populations were highest in all cases in the groups 'under 4 mm.' and '4 mm.', and became smaller with increasing wireworm lengths. This distribution is typical of wireworm populations whose pyramidal form was first demonstrated by Salt & Hollick (1944). The wireworms over 15 mm. in length were not included in the figure because of their small numbers. The fluctuations in numbers occur chiefly in the groups 'under 4 mm.', '4 mm.' and '5 mm.', and the seasonal changes noted in Fig. 1 are due mainly to these smaller sizes.

The total wireworms of length less than 4 mm. recovered during the investigation was 6595, and of length '4 mm. and over' was 20,702. In other words, the wireworms 'under 4 mm.' constituted 24·2% of the wireworms recovered throughout the year.

(*d*) *Changes in the wireworm populations in particular fields.* Although the wireworm populations of all six fields showed the definite seasonal fluctuation already noted, differences can be detected in particular fields. Thus, in field I (Fig. 1)

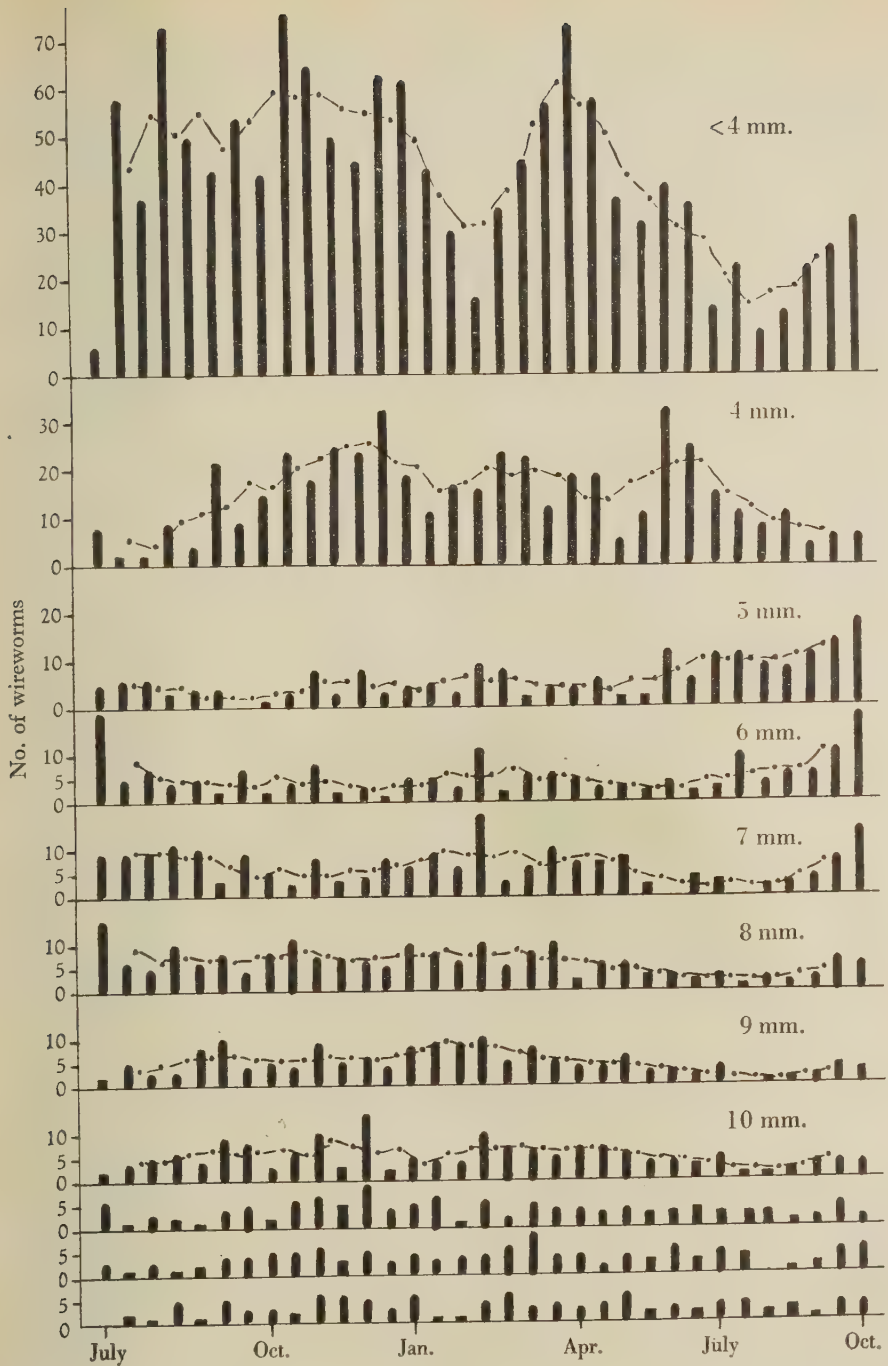

Fig. 2. Numbers of wireworms of different lengths at each sampling of field I.
— · — · — Running mean.

a marked decline occurred in total population from late December 1943 until mid-February 1944, followed by an increase to a peak in April. From Fig. 2 it can be seen that this fluctuation was due almost entirely to the population changes in the wireworms 'under 4 mm.'

In field III (Fig. 1) a relatively low population was recorded in the latter part of December 1943, which gave the running mean the appearance of a double peak as in field I. In July 1944 a sudden influx of wireworms 'under 4 mm.' was recorded to reach the highest figure for this group throughout the investigation. However, subsequent estimates reverted to the low populations found in most samples taken during the summer months.

Field V was ploughed during the first week of July 1944 and was sampled a fortnight later. The population showed a sharp rise in numbers to reach the highest total recorded for this field. This increase was not maintained, however, and as in field III in August the lowest population estimate for any sampling of this field was recorded.

It is worth noting that the fields adjacent to fields I, III and V did not show changes corresponding to those just described.

(*e*) *Changes in depth distribution.* Sampling to depths below 6 in. was done in the usual way. No special modifications were introduced to prevent wireworms falling in from the sides of the hole, so it must be assumed that our figures for wireworms below 6 in. are in excess of the actual numbers present. However, this is one of the errors inherent in sampling and probably also occurs in samples taken to a depth of 6 in. only.

The wireworms recovered throughout the investigation at the various depths are shown in Figs. 3*a* ('4 mm. and over') and 3*b* ('under 4 mm.').

0–6 *in. fractions.* The seasonal fluctuation seen in the total population in Fig. 1 is also seen in the wireworms '4 mm. and over' in the 0–6 in. fraction. In all six fields the lowest estimates were recorded in July and August in both 1943 and 1944, and the peak populations occurred between January and April. This fluctuation occurs also in the wireworms 'under 4 mm.', although it is less pronounced.

Some features in the results on individual fields can now be analysed. The dip in total population on field I (Fig. 1) from December to mid-February is clearly due to a decrease in the population in the 0–6 in. fraction, amounting to a drop of about 1,000,000 wireworms per acre in each of the categories 'under 4 mm.' and '4 mm. and over'. In field III the low population recorded in late December 1944 was due mainly to a decline in the population of wireworms '4 mm. and over' in the 0–6 in. fraction.

The very high population recorded from field III in July 1944 is now seen to have been due to the sudden appearance of wireworms 'under 4 mm.' in the 0–6 in. layer. On the other hand, the increase in population in field V after ploughing in July 1944 occurred in both the categories 'under 4 mm.' and '4 mm. and over'.

6–12 *in. fractions.* The seasonal fluctuation in population is less clearly marked

in the 6–12 in. fractions. However, in fields I–V there is a slight suggestion that the trend is reversed as the populations are higher in July and August and lower in January to March, the period of the peak populations in the 0–6 in. fraction. In field VI the numbers in the 6–12 in. fraction behaved more like those of the 0–6 in. fraction, showing a peak in the winter months in both categories.

12–24 *in. fractions.* The populations in these fractions were recorded from fields I, II and VI only. Except in field VI the numbers of wireworms collected are small, but it is evident that higher populations occurred in July and August than in January to April. As would be expected, fewer wireworms were recorded as the sampling depth increased. It is only in field VI that the number of wireworms allows us to state that the winter populations below 12 in. were significantly smaller than the summer populations.

In all the fields there was a marked increase in the population of wireworms in the 0–6 in. layer from July 1943 to March and April 1944, accompanied by a less marked decline in population in the deeper layers of the soil. From April to August 1944, the population of the 0–6 in. layers declined sharply, but there was no evidence of a corresponding increase in the population of the deeper layers.

The total number of wireworms recorded at each 6 in. horizon throughout the year is shown in Table 1. In the category 'under 4 mm.' over 75 % were found in the

TABLE 1. *Distribution of wireworm population in depth*

Field	Total 0–6 in.	Total 6–12 in.	Total 12–18 in.	Total 18–24 in.	% 0–6 in.	% 0–12 in.
			Wireworms less than 4 mm. length			
I	1,064	309	14	5	76·4	98·6
II	927	184	24	2	81·5	97·8
III	1,085	293	—	—	78·7	—
IV	470	158	—	—	74·8	—
V	637	148	—	—	81·2	—
VI	575	568	107	25	45·1	89·6
Total	4,758	1660	145	32	72·6	—
			Wireworms 4 mm. and over			
I	2,118	534	131	26	75·4	94·4
II	2,311	547	133	30	76·5	94·6
III	2,533	738	—	—	77·6	—
IV	2,011	427	—	—	82·5	—
V	2,796	459	—	—	85·9	—
VI	3,410	1733	507	238	57·9	87·3
Total	15,179	4438	771	294	73·4	—

0–6 in. layer, except in field VI, where rather less than half occurred. The mean proportion was 72·6 %. In the category '4 mm. and over', 75–85 % of the population occurred in the 0–6 in. layer except in field VI, where the proportion was 57·8 %. If only fields I, II and VI are considered, it will be seen that approximately 90 % of the population occurred in the 0–12 in. layer. It would appear that for most experimental purposes, sufficient information can be obtained if sampling is confined to a depth of 12 in.

Discussion

The recorded rise in population from a minimum in July–August to a maximum in January–April is contrary to what would be expected from the life history of *Agriotes* spp. as described by numerous workers (Roberts, 1919; Cohen, 1942; Evans, 1944). It is generally agreed that egg-laying takes place in May and June and that larvae hatch out in about 4–5 weeks. After a larval life of 4–6 years (Evans, 1944) pupation takes place in July and August. The new adult generation spends the winter in the soil and reproduces in the following spring.

In accordance with this life history the total population should reach a maximum in July or August due to the hatching of new individuals. During the rest of the year the population should decline as a result of mortality, and eventually pupation of some of the *Agriotes* larvae in the field. Within the size groups we should expect a sudden influx of larvae 'under 4 mm.' in July followed by a decline in this group as the effects of mortality and growth become apparent. The larvae '4 mm. and over' should show corresponding changes indicating the influx of wireworms from smaller size groups and loss to the larger size groups. It is obvious that our figures show these expected trends only in the latter part of the year, and that we cannot interpret the period from July to January on any of our six fields in terms of the accepted life history.

There are no published data on wireworm populations in grass fields with which our data can be compared. Salt & Hollick (1944) have published results of repeated samplings of one grass field in the form of percentages occurring in the different size groups, but have not stated the actual numbers of wireworms recovered. They have also used smaller units (0·4 mm.) in measuring larval length. Consequently we cannot detect the seasonal changes within the size groups which they have reported.

The anomalous rise in population that we have observed from summer to winter months immediately throws doubt on the reliability of our figures. If, for any reason, from the time of hatching in July up to the following January, an increasing proportion of the larvae in the new generation appeared in the counts, the records would show just such an increase in the total population. This would happen, for example, if the transformation from delicate to more robust larvae led to a more complete extraction.

The possibility that our counts are incomplete makes it necessary to consider once again the efficiency of the extraction process.

The tests described in the previous paper (Cockbill *et al.* 1945) were carried out on wireworm '4 mm. and over' and on *Tenebrio* larvae of all sizes including some 'less than 4 mm.' We have tried, therefore, to supplement earlier work on this point by testing specifically the efficiency of the apparatus in extracting newly hatched larvae in the summer months. These additional tests were of two kinds: (1) tests on *Agriotes* larvae hatched out from eggs in the laboratory; (2) tests on the

effect of modifying the extraction technique on sets of samples taken simultaneously from a single field.

(1) *Tests on newly hatched larvae*

Within a few days of hatching, larvae were subjected to treatments corresponding to the different stages in the extraction technique and the effects observed. By placing groups of five larvae in glass tubes with soil and water it was found that soaking for periods up to 7 days did not cause any noticeable changes in the appearance of the larvae. Many of the larvae remained alive during the treatment, and those which died showed no sign of decay and appeared to be as tough and firm as at the beginning of the test. It was found also that these larvae floated readily in brine solutions of sp.gr. 1·10–1·14. Larvae treated in this way were boiled for 1 hr. When a layer of paraffin was added, stirring brought all the larvae to the interface where they could be picked off without difficulty. These tests indicate that there is no reason to suspect that newly hatched larvae are lost at any of these stages in the process. The only point which could not be tested in this way was the possibility of destruction by the jet of water during washing, but other tests to be described throw light on this question.

Three tests on the recovery of newly hatched larvae from wireworm-free soil were also carried out. The soil was taken from the residues of previous washings with grass and grass roots added to make the sample resemble an ordinary sample as closely as possible. Forty larvae were placed in each of three tins containing soil, allowed to stand for 1 day and soaked for 2 days. Then the samples were extracted in the usual way without any special precautions. In the three samples 36, 38 and 38 of the larvae were recovered, showing that 8 out of 120 or 6·6% had been lost. The examination of residues showed that in the first test two larvae had been caught up with the grass roots during washing, but the remaining six larvae were not found in the residues. This result indicates that the level of efficiency of the apparatus (95%) deduced from earlier tests applies also to newly hatched larvae. There can be little doubt, therefore, that the apparatus, as used in routine work, is capable of extracting most of the small *Agriotes* larvae that would be expected to occur in the samples in the summer months.

It is worth adding that the wireworms extracted in these recovery tests were recovered in good condition and showed no sign of damage caused by the high-pressure jets used in the washing process. The tests have also shown that newly hatched larvae do not pass through the apertures of gauze of 60 meshes/in. to any appreciable extent. This confirms our earlier conclusions on this point as a result of tests with sieves fitted with gauze of 60 and 100 meshes/in. (Cockbill *et al.* 1945).

(2) *Tests with modified extraction technique*

The object of these tests was to see if it was possible, by modifying the technique in various ways, to obtain the peak population, especially of newly hatched larvae, that would be expected in the summer months. Accordingly, nine sets of twenty

samples each were taken from a single field on each of two occasions in July 1945. The treatments were designed to show whether large numbers of small wireworms might be lost in any of the following ways: (*a*) decay during soaking; (*b*) destruction by high-pressure jets; (*c*) imprisonment in residual lumps of soil; (*d*) destruction by boiling. Thus in one group of samples the soaking time was varied, in another boiling was omitted or reduced; in a third group the residual lumps were broken down into finer particles and a fourth group was washed with low-pressure jets. We have already noted that in estimates based on twenty samples the sampling variation is considerable, and therefore it was to be expected that the results would fluctuate between wide limits. Nevertheless, the results showed plainly that the elimination of factors (*b*), (*c*) and (*d*) above did not lead to the extraction of higher numbers of larvae. We have enough data on (*a*) to consider this point in some detail.

Table 2

Days soaked	Wireworms recovered		Total
	Less than 3 mm.	3 mm. and over	
2	30	81	111
3	68	83	151
3	40	62	102
4	38	77	115
5	27	61	88
6	24	48	72
7	29	53	82
8	13	67	80

Table 2 shows the number of wireworms 'less than 3 mm.' recovered from sets of samples soaked for periods from 2 to 8 days. The samples were taken on the same day, and except for the variable soaking factor were treated alike in the laboratory. The table clearly suggests that the counts tend to fall when the period of soaking exceeds 4 days. It happens, however, that records were kept of the duration of soaking in all our routine wireworm extractions. Analysis of these records does not support the tendency apparent in Table 2, and suggests strongly that the soaking factor cannot be responsible for the anomalous differences between the summer and winter populations. In the first place, the records show that samples were seldom soaked for more than 5 days in the course of this work. Secondly, the counts recorded on individual fields throughout the year show no tendency to vary with the duration of soaking, and some of the highest estimates were obtained from samples soaked for more than 5 days. Thirdly, the records show that the average soaking times for the six grass fields were longer in the winter than in the summer months (July–August 2·7 days; November–December 4·0 days; January–February 3·7 days). In view of these facts and the observations on the effect of soaking newly hatched larvae described above it seems unlikely that the low populations recorded in the summer are due to the decay of the larvae during soaking.

In our opinion these additional tests have dealt with those factors in the extraction technique which might have accounted for the anomalous rise in population from

summer to winter. Therefore, it is necessary to seek the explanation elsewhere, probably in the biology and behaviour of *Agriotes* under field conditions. On this matter we have little to add to existing knowledge, but it may be useful to dwell briefly on some of the possibilities.

It has been suggested at various times by farmers and others that wireworms penetrate to the deeper layers of the soil during certain periods of the year and thus are not recorded in the sampling. The effect of this downward migration would be to indicate an apparent decline in total population. Conversely, the apparent increase in the populations from August to April might be due to an upward migration of wireworms from the deeper layers. If such a movement were to take place some evidence of it would have been noted in this type of investigation. For example, in fields I, II and VI (Fig. 3) the populations increased from August to March and April in the 0–6 in. layer without a decline in the population in the deeper layers sufficient to account for it.

TABLE 3. *Population changes in relation to depth. Total wireworms (running means)*

	Constitution of lowest population recorded in summer 1943		Constitution of highest population recorded in spring 1944	
Field	Population 0–6 in.	Population below 6 in.	Population 0–6 in.	Population below 6 in.
I	72·3	46·0	126·8	26·5
II	69·8	38·8	137·0	27·0
III	51·8	47·0	159·3	32·5
IV	48·5	25·8	123·0	19·5
V	66·5	35·5	187·3	30·3
VI	78·8	158·8	190·5	127·8
Mean	64·6	58·6	154·0	43·9

Mean increase in population 0–6 in. = 89·4.
Mean decrease in population below 6 in. = 14·7.
Net increase in population 74·7.
Maximum contribution to increase from below 6 in. = 16·4 %.

Table 3 shows the mean increase in population of the 0–6 in. layers based on the running means from August to April compared with the decrease in population in the deeper layers for the same period. Thus in field I the lowest mean population recorded in the summer of 1943 was 118·3 wireworms, of which 72·3 occurred in the 0–6 in. layer and 46·0 in the deeper layers. In the spring of 1944 the highest mean population was 153·3 wireworms, of which 126·8 occurred in the 0–6 in. layer and 26·5 in the deeper layer. Taking the mean of the six fields, the population of the 0–6 in. layer increased from 64·6 to 154·0, a net increase of 89·4, while the decrease in the deeper layers was from 58·6 to 43·9 or 14·7 wireworms. Thus only 16·4% of the apparent population increase in the 0–6 in. layer could be due to an upward migration of wireworms from the deeper layers. The difference of about seventy-four wireworms must represent the influx of new individuals into the counts from other sources.

A more likely possibility is that under field conditions in the summer the newly hatched larvae enter the plant roots in large numbers. This would explain their failure to appear in the samples at this time. Their appearance in later months

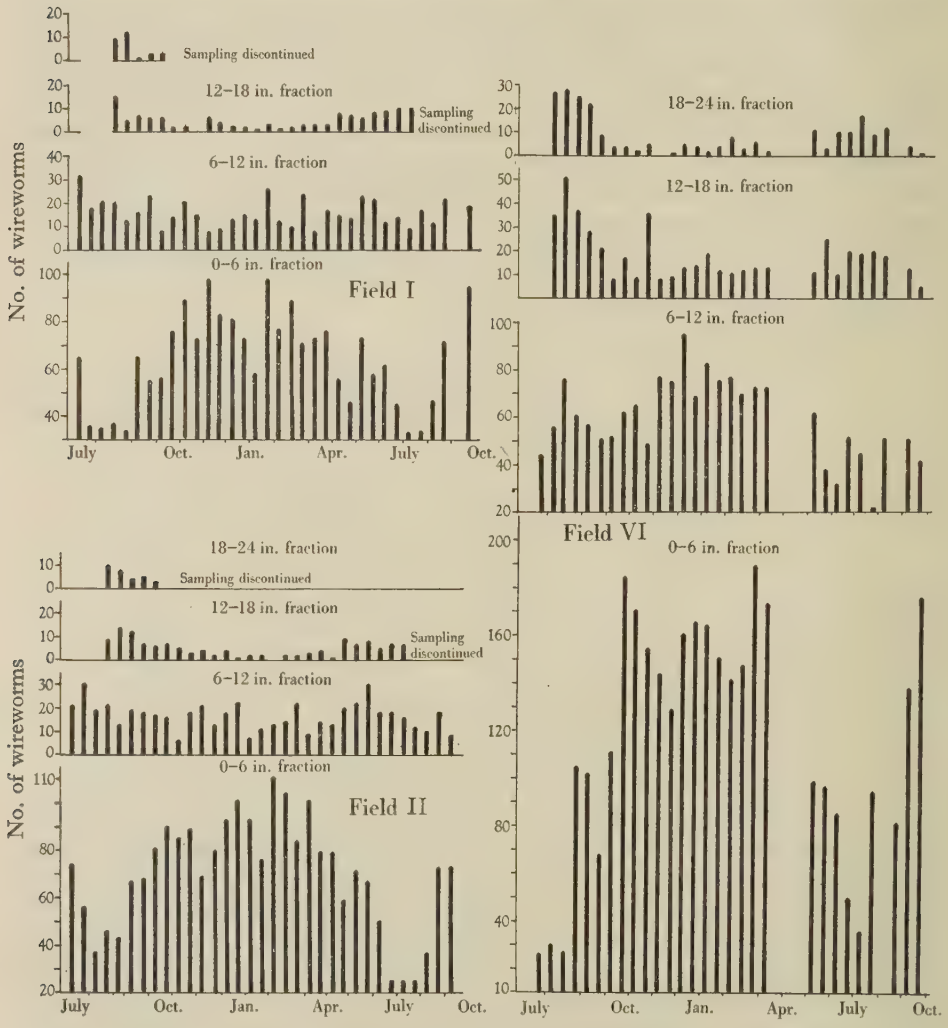

Fig. 3*a*. Depth distribution of wireworms 4 mm. and over in length. Fields I, II and VI.

would then be due to their emergence from the roots with increase in size. This point is being investigated. If young larvae behave in this way, however, an almost insurmountable difficulty will be introduced into the problem of extracting samples of the complete wireworm population.

A third possibility is that hatching of the wireworm eggs laid in May and June is spread over several months so that the new individuals are added gradually to the existing population. This seems unlikely for a number of reasons. First, all workers who have reared larvae from eggs in the laboratory have reported that most of the eggs hatch within 4–5 weeks. Our own tests have shown that few eggs remain after

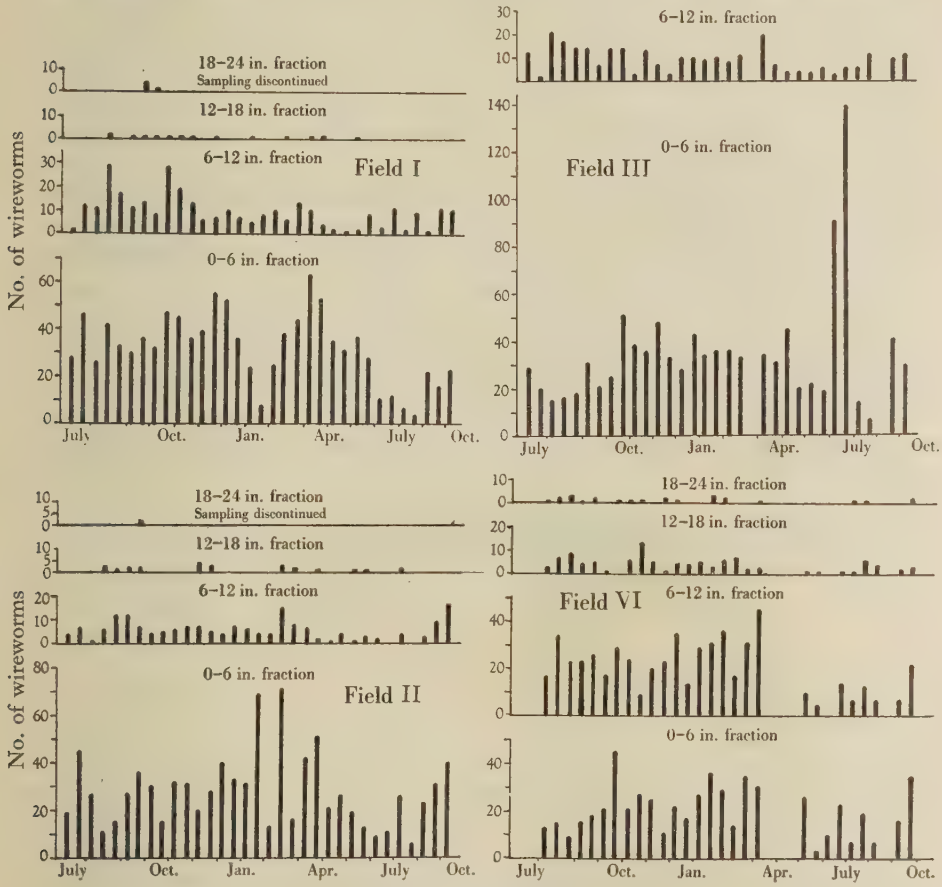

Fig. 3*b*. Depth distribution of wireworms less than 4 mm. in length. Fields I, II, III and VI.

this hatching period and these soon disappear. Secondly, our experience in rearing wireworm larvae from eggs in the laboratory suggests that the eggs readily dry out and die. If hatching were spread over several months in this way, the eggs would be required to remain alive in the surface layers of the soil during the driest and hottest period of the year.

There is also the possibility that the adults emerging from pupation initiate

a second period of egg-laying in the autumn. Such behaviour occurs commonly in other insects, but there is no evidence for it in *Agriotes*. Cohen (1942) has stated that the adult remains inactive in the pupal cell below the surface of the soil until the following spring. Moreover, if two reproductive periods occurred every year, we should expect to find two peaks in the population in summer and autumn corresponding to the two hatching periods. This does not occur, nor is there any marked influx of larvae 'less than 3 mm.' into the counts in the autumn months (cf. Table 4). We have tested this point by examining adults collected in August and September.

Finally, it should be noted that most wireworm populations consist of mixtures of the three species of *Agriotes*: *A. lineatus*, *A. obscurus* and *A. sputator*. It might be possible to account for the observed rise in population if differences existed between the reproductive behaviour of different species. Laboratory breeding experiments have not revealed any such differences. It is perhaps worth noting, however, that field VI, which showed the greatest difference between summer and winter populations, contains a population consisting almost entirely of *A. sputator*.

Although the occurrence of minimal populations in the summer months is the most striking feature of our results, it would be incorrect to say that no influx of small wireworms occurs at the expected time. Table 4 shows a marked increase in the number of wireworms 'less than 3 mm.' in July corresponding to the period of hatching.

Table 4. *Monthly records of wireworms of length less than 3 mm. from grass fields in* 1944

Month	Total	As % total wireworms recorded per month	As average per field
March	176	2·8	9
April	126	5·0	8
May	100	2·1	5
June	21	0·5	3
July	434	11·8	15
August	180	5·6	8
September	65	3·0	3
October	37	1·8	3
November	30	—	—

The number of new individuals appearing in July represents an increase of only about 300,000 per acre in the field populations. This is far too small to account for the numbers of older larvae commonly found in grass fields assuming that considerable mortality occurs and that the larvae have a life of 5 years. It is noteworthy, however, that by treating the results as percentages in this way, the same trends reported by Salt & Hollick (1944) for wireworms 2·0–2·8 mm. in length become apparent. But since this treatment masks the rise and fall in absolute numbers of wireworms we believe it gives a somewhat misleading picture of the seasonal changes observed.

Another point of interest in Table 4 is the considerable number of wireworms less than 3 mm. in length that were recovered in the months March–May, almost a year after the recognized hatching period. This does not necessarily mean that new individuals are being added to the populations at this time, as larvae vary considerably in their rates of growth (Evans, 1944). Also, increase in length is by no means a reliable criterion of larval growth. Nevertheless, the figures suggest that first-year larvae are appearing in the samples for the first time during this period. Possibly the appearance of these larvae and the anomalous increase in population that we have been discussing are two aspects of the same phenomenon.

It is evident that only tentative conclusions can be drawn from the results of this investigation. Until they are confirmed by other methods or satisfactorily explained by new data they cannot be advanced as a complete picture of the annual changes in wireworm population in permanent pasture. It is necessary, however, to recall the original purpose of the investigation. This was to provide controls against which the behaviour of wireworm populations in cultivated fields could be studied. Although it is a serious disadvantage when the controls vary in a manner that cannot be explained, yet the comparison may be useful. Therefore, it is simply as a background for our concurrent studies on populations in cultivated fields that these results are presented here.

There is one practical consideration arising from this work which should be mentioned. This is the fact that populations estimated in this way show big seasonal fluctuations over and above the statistical variation. This means that single estimates of wireworm populations are of doubtful value in predicting wireworm damage or in comparing the effects of different treatments and conditions. In such work a series of estimates is desirable, and the time of year at which the estimates are made must be taken into consideration.

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WIREWORM POPULATIONS IN RELATION TO CROP PRODUCTION

III. POPULATION CHANGES AFTER SUMMER PLOUGHING

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(With 4 Text-figures)

Six permanent pastures broken up in the summer of 1943 were sampled once a fortnight after ploughing in order to study changes in the wireworm populations.

The graphs of the running means of four consecutive counts showed the chief trends in population. On four of the fields, a slight increase in population was apparent up to December. Only in February and March was a definite decline apparent. On the other two fields, the counts declined by about 30% during the autumn and winter.

A marked fall in population (from 30 to 70%) was evident on all the fields only after the first crop had been harvested. The new population at this time was characterized by an absence of larvae less than 4 mm. in length.

The results considered alone indicate that after summer ploughing the wireworm population remains unaffected until the following spring and summer during the cropping period. When the results are considered in relation to the grass 'controls', it appears that a net decline in population occurs during the autumn and winter and that the decline in spring and summer is not more marked than that which normally occurs at this time in grass fields. Unlike the grass fields, however, the reduction is permanent.

Introduction

It is common knowledge that the ploughing and cropping of grassland brings about a decline in wireworm populations. After several years of arable cropping, fields that were originally permanent pastures usually contain negligible numbers of wireworms and are assumed to be safe from the danger of wireworm attack. But it is not known when the decline occurs or what factors are chiefly responsible for bringing it about. If the answers to these questions were known, they might indicate the measures that could be taken to reduce the wireworm population in the shortest space of time.

The wartime wireworm survey in England and Wales yielded some information on the size of the reduction in the populations that occurs during the first two years of cropping ploughed-up grassland. The percentage reduction depends on the initial population, being much greater on heavily infested fields. Thus the populations on fields with 2,000,000 wireworms per acre were reduced by 63% after 1 year and by 87% after 2 years. At the other end of the scale, populations on fields with 250,000 wireworms per acre were reduced by 10% after 1 year and 50% after 2 years

(*Bull. Minist. Agric.* no. 128, 1944). The data collected in the wireworm survey, however, must be treated with reserve for a number of reasons. The counts recorded in the survey were all based on the wireworms recovered from the soil by hand-sorting. It is now known that this method fails to recover constant proportions even of the older larvae and misses almost all larvae less than 5 mm. in length (Salt & Hollick, 1944; Cockbill *et al.* 1945). Therefore, the figures quoted above do little more than confirm the belief that populations decline when grass is ploughed up and cropped. They provide only a partial answer to the question of the magnitude of this decline, and they tell us nothing at all as to what stage or stages in the process produced the decline or how the different age groups of larvae were affected.

Our investigation was planned as an intensive study of the effect of summer ploughing followed by winter or spring cropping on the wireworm populations in a few selected fields. We hoped that sampling these fields at frequent intervals throughout the year would show the sequence of events under these conditions. As we had available an improved method for recovering the larvae from the samples we also hoped to gather data on the effects of ploughing and cropping on the different age groups. However, the labour demanded for such a project limited it to a few fields, and therefore the results obtained might not be applicable to all fields under these conditions.

In the preceding paper we have described the results on what were intended originally to be the 'controls' for this investigation, a series of grass fields sampled throughout the same period (Ross *et al.* 1947). Because these 'controls' showed an unexplained annual fluctuation, we have treated them separately. In spite of this difficulty of interpretation, we believe the results of this investigation should be placed on record as the first attempt to find out precisely what is the effect of breaking up grassland on the wireworm population.

Methods

The sampling and extraction techniques described in preceding papers were used in this work. On each occasion, twenty standard samples scattered over the field were taken and the wireworms extracted from them bulked in two lots of ten samples by washing followed by brine and paraffin flotation. Tests described in earlier papers have indicated that this method of extraction yields about 95% of the larvae in the samples. All samples were taken to a depth of 12 in., and those from the 0–6 and 6–12 in. layers were treated separately in order to provide information on the vertical distribution of the wireworms.

Certain problems arise in sampling ploughed fields which are not present in work on grass fields. A grass field can be regarded as a fairly uniform whole, and any one of the samples taken from it will be roughly similar in size and compositon. In a ploughed field this uniformity is lost and samples vary enormously in the amount of soil or turf they contain, and in the physical condition of the soil. Ploughing also upsets the basis of random sampling to some extent. Especially in fields that

are unevenly ploughed with furrows at various angles with the horizontal, some selection of sampling points is forced upon the sampler. Apart from these statistical problems there is the fact that as a result of the loosening of the soil caused by ploughing, samples of soil from ploughed fields tend to contain less soil than samples taken to the same depth on grass fields. In some cases, however, the same factor may tend to increase the amount of soil in the samples as the loosened soil tends to fall in from the edge of the hole during sampling, especially under dry conditions. Of all these problems, the last is the only one that we attempted to eliminate. A metal sleeve slightly wider than the sampling tool was driven into the ground before the sample was taken to prevent the edge of the hole from collapsing. However, this so increased the labour involved in sampling that it was used only in the most difficult cases.

Results

Six grass fields in Huntingdonshire scheduled for ploughing during the summer of 1943 were selected for the investigation.* These will be denoted fields VII–XII to distinguish them from fields I–VI of the preceding paper. Fields VII, VIII, X, XI and XII were situated in shallow valleys drained by the Alconbury Brook system, and their soils were classified as silt overlying boulder clay. Field IX was situated on higher ground with a high proportion of clay in the surface layer.

The fields were first sampled in July 1943 when they were still in grass. Each field was sampled a second time not later than a fortnight after ploughing and subsequently at fortnightly intervals until the growth of the crop in April prevented further sampling. Fields IX, X, XI and XII were ploughed in July, but fields VII and VIII were not ploughed until late September.

The results are set out in the same way as those on the permanent grass 'controls' in the preceding paper. Thus Fig. 1 shows the wireworms collected at each sampling and the running means of four counts of wireworms 'under 4 mm.', '4 mm. and over' and 'total' as curves. The dates of ploughing and the periods when the growing crops prevented sampling are indicated for each field.

It will be seen that the fields differed widely in their original populations, ranging from 2 to 5 million wireworms per acre. It is unfortunate that more estimates of the populations in grass are not available, but in all except two cases the fields were ploughed a few days after they were selected for the investigation.

The wireworm counts in Fig. 1 show two types of variation: (*a*) short-term fluctuations often between successive estimates; (*b*) long-term trends in population.

(*a*) *Short-term fluctuations*

The short-term fluctuations are undoubtedly a statistical effect due to the considerable error inherent in counts based on twenty samples. It might be expected that owing to the less uniform conditions after cultivation, these counts would show

* Field VII: Alconbury 210. Field X: Great Stukeley 260.
Field VIII: Leighton 118. Field XI: Little Stukeley 2.
Field IX: Leighton 154. Field XII: Spaldwick 84.

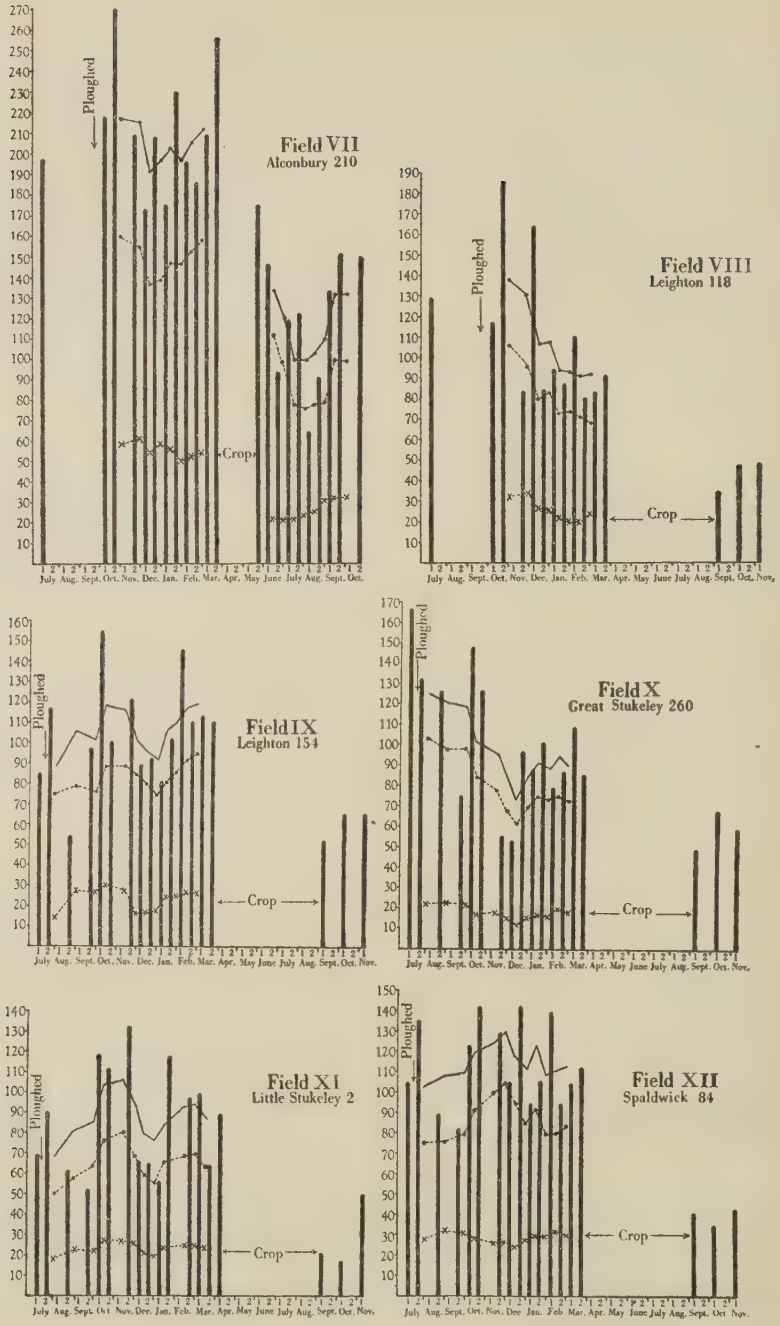

Fig. 1. Fields VII–XII. Wireworm numbers at successive samplings.

| Total wireworms.
——— Running mean of total wireworms.
-·-·- Running mean of 4 mm. and over.
-×-×- Running mean under 4 mm.

this type of variation to a greater extent than those of the grass fields. However, comparison between these diagrams and those in the preceding paper shows that this is not so, and in fact, most of the counts lie reasonably close to the mean. This view that counts based on twenty samples are equally reliable for grass and ploughed fields is borne out by an analysis of variance in the number of wireworms found in single samples in the two cases. As in the preceding paper, however, we can regard as significant only population changes of considerable magnitude and that occur over a series of estimates. Such changes are brought out clearly in the running means.

It is interesting to compare the estimates obtained in grass with those immediately after ploughing. Of the four cases where ploughing took place soon after the single estimate of the population in grass was obtained, only one gave a lower count after ploughing. In general, however, the differences were small. It seems unlikely, therefore, that ploughing has any immediate effect on the population.

(*b*) *Long-term trends in population*

The trends in population up to March 1944, when the sampling was interrupted, are shown by the running means in Fig. 1. The form of the curve varies from field to field and we shall have to discuss whether these are variants of the same essential form or whether they represent different trends reflecting different conditions.

One feature may be observed which is common to all the fields. This is the marked dip in the curve occurring about the beginning of December. Except in field VIII this is a temporary phase and in most cases the populations are close to the pre-December level by February. It is worth recalling that the grass fields I and III showed a similar dip at this time. Evidence will be produced later which suggests that the dip coincides with a downward movement of wireworms in the soil.

Apart from field X, the curves may be reduced to a common form rising to a peak in the autumn. This is seen clearly in fields IX, XI and XII. In fields VII and VIII where ploughing was delayed until September, the same tendency is apparent in the October counts. Field X, on the other hand, shows a steady decline throughout the autumn.

When these curves are compared with our grass field 'controls', a number of points emerge. The type of curve obtained on fields IX, XI and XII, rising to a peak in the autumn and followed by a dip in December and January, is essentially similar to the grass field curve during the first part of its course. After the dip, which occurs only on two of the 'controls', the first signs of a decline in population become apparent but at most this effect is slight and the final counts in March still exceed those of the preceding summer. Fields VIII and X, on the other hand, differ from the 'controls' in showing a fairly steady decline in population throughout (except for the recovery after the dip in field X). By March the counts on these fields are 32 and 28% below the first points on the curves. Field VII differs from the other fields, its mean populations remaining almost constant. But this field had a different history, which

probably accounts for its behaviour. It was ploughed and disked in late September and drilled with wheat. The turf was not killed by the ploughing and the grass continued to grow amongst the wheat plants.

It is clear from these results that the ploughing of grass from July to late September has remarkably little effect on the wireworm population. This is probably true whether the field is left fallow during the winter with occasional cultivations or sown with a winter crop. If the population declines, it does so gradually and the final reduction is not large. But it appears to be more usual for the counts to show changes resembling the seasonal trends that we have already observed in grass fields and that we have been unable so far to explain. Where such changes occur in ploughed fields, however, the numbers of wireworms involved are considerably smaller than those on most of the 'controls'.

Although ploughing at these times does not have any marked effect in reducing the populations, comparison with the grass fields suggests that the counts on the ploughed fields are probably lower in March than they would have been had the fields remained in grass. During this month we have found that counts from grass fields were usually maximal, sometimes even being twice as large as the counts obtained during the previous summer. Therefore, it can be said that the chief result of ploughing in the summer is to damp down and sometimes cancel out the anomalous rise in wireworm counts recorded in our study on grass fields.

In considering the later history of the fields and the effects of cropping, field VII must be treated separately. This field was drilled with winter wheat in October 1943 soon after ploughing. In spite of the high wireworm population and inadequately prepared seedbed, the wheat germinated well and appeared healthy throughout the winter and early spring. In May, however, growth ceased and wireworm damage appeared on an extensive scale. Eventually the crop was a complete failure, evidently from manurial deficiencies coupled with wireworm damage. It was possible, therefore, to resume sampling on this field in May. The results (Fig. 1) showed that a considerable reduction in population had occurred in April and May and that this tendency continued until July, after which time the counts rose again until October when sampling was discontinued. The latter effect is probably due to the fact that the grass had never been killed, and after the wheat failed the grass became re-established and the field showed the counts rising from a minimum in July which is typical of grass fields.

Sampling was resumed on fields VIII–XII in September 1944 after the crops had been harvested. Three estimates of the population were taken on each field. In every case, a marked reduction in population had occurred in the course of the summer, although the decline varied considerably from one field to another. On field X the reduction recorded from March to September was about 30%, on fields VIII and IX about 50% and on fields XI and XII about 70%. This is in keeping with the figures presented in an earlier paper of this series (Cockbill *et al.* 1945) on the decline in population during the first year of cropping. It is noteworthy that

the counts recorded were not in every case the lowest for the respective fields, e.g. fields IX and X. It is clear that the size of the reduction depends on the factors operating in each field.

There is no obvious explanation for the differences between fields VIII–XII in this respect. Fields X and XII, which fell by 30 and 70% respectively, were both cropped with barley. The other fields were cropped with peas. Cultivations on all five fields were similar, ploughing followed by disk harrowing two or three times during the autumn and winter. Moreover, the differences are not due to a tendency for the reduction to vary with the original infestation (*Bull. Minist. Agric.* no. 128, 1944) since the mean populations of all the fields in March were between 2 and 3 millions per acre. Clearly, the conditions under which the decline in population occurs during the cropping season are too complex to be sorted out by this type of investigation.

The results on all six fields show distinctly that the first marked fall in population following the breaking up of grass occurs during the months March to September. It must be remembered, however, that the populations on our grass field 'controls' also declined during the period March to July. It would be incorrect, therefore, to suggest that the observed decline in population is entirely a result of the arable state of the fields, since it would have occurred to some extent even if the fields had remained in grass. But the decline is obviously much greater on the ploughed fields and results in a permanent reduction in the population to a much lower level.

(*c*) *Changes within the size groups*

In Fig. 1 the running means are drawn for wireworms 'under 4 mm.' and '4 mm. and over'. In general, the curves are parallel to each other and to the curve for total wireworms. The only exceptions to this general rule are in field VII, where the upward trend from December to March is not reflected in the wireworms 'under 4 mm.' and in field XII where the decline in January occurs only in the wireworms '4 mm. and over'.

Fig. 2 shows the numbers of wireworms of different lengths recovered from fields IX and XI throughout the year. In all cases except the first sampling in July 1943 there is a preponderance of larvae less than 4 mm. long. This situation is radically altered as a result of cropping and very few larvae of this size group were recovered in September–November 1944. Although a reduction in numbers is evident in all the other groups, it is clear that the main reduction occurs in the small forms and reflects the absence of the normal annual influx of newly hatched individuals into the population. At the same time it can be seen that probably more than 50% of the young larvae added to the population in 1943 (roughly represented in the March 1944 counts by the groups 5 mm. and under) have failed to survive the period of cropping. It is difficult to arrive at any definite conclusions about the numbers of larger larvae remaining because their numbers are so small and variable. The figures indicate that some decline has taken place in all groups of wireworms

above 5 mm. and that this decline is more marked in field XI than field IX. The other fields, for which the figures are not shown, exhibit the same trends, except field VII on which grass became re-established and on which an influx of wireworms 'less than 4 mm.' was evident in 1944.

Comparison between the ploughed and grass fields throughout the year shows that on the former there is no pronounced increase in the numbers of smaller larvae in the counts from July 1943 to April 1944 as in the grass fields. It is interesting to note, however, that of the 9242 larvae recovered throughout the investigation, 2644 (22·5%) were larvae less than 4 mm. in length, a figure very close to the proportion (24·2%) on the grass fields.

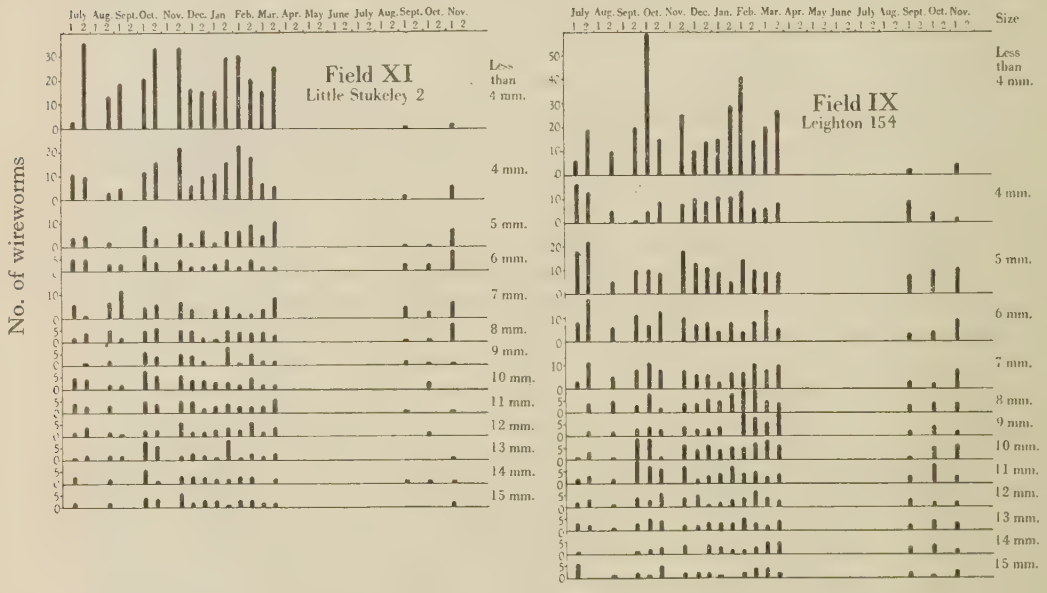

Fig. 2. Number of wireworms of different lengths at each sampling of fields XI and IX.

(*d*) *Changes in depth distribution*

In the grass fields about 75% of the wireworms were found in the 0–6 in. layer of the soil. Since ploughing turns this layer upside down or on its side, it would be expected to alter the vertical distribution of wireworms very considerably. Table 1 shows the wireworms recorded in soil from the 0–6 and 6–12 in. from all fields throughout the investigation. It is clear that the fields vary greatly in this respect. Probably this reflects differences in treatment or conditions on the different fields. On the average, however, the wireworms tend to be equally distributed between the two layers, though individual fields may have from one-third to two-thirds of the larvae in one of the two layers. In general, the distribution of wireworms 'less

than 4 mm.' and '4 mm. and over' is similar. In fields IX and XI, however, the small forms are concentrated in more than average numbers in the 6–12 and 0–6 in. layers respectively.

TABLE 1. *Distribution of wireworm population in depth*

	Wireworms less than 4 mm. length			Wireworms 4 mm. and over		
Field	Total 0–6 in.	Total 6–12 in.	% 0–6 in.	Total 0–6 in.	Total 6–12 in.	% 0–6 in.
VII	540	410	56·8	1731	1182	59·4
VIII	145	146	49·8	599	483	55·4
IX	84	247	25·4	560	869	39·2
X	131	165	44·3	660	748	46·9
XI	250	87	74·2	665	372	64·1
XII	122	317	27·8	460	913	33·5
Total	1272	1372	48·1	4675	4567	50·6

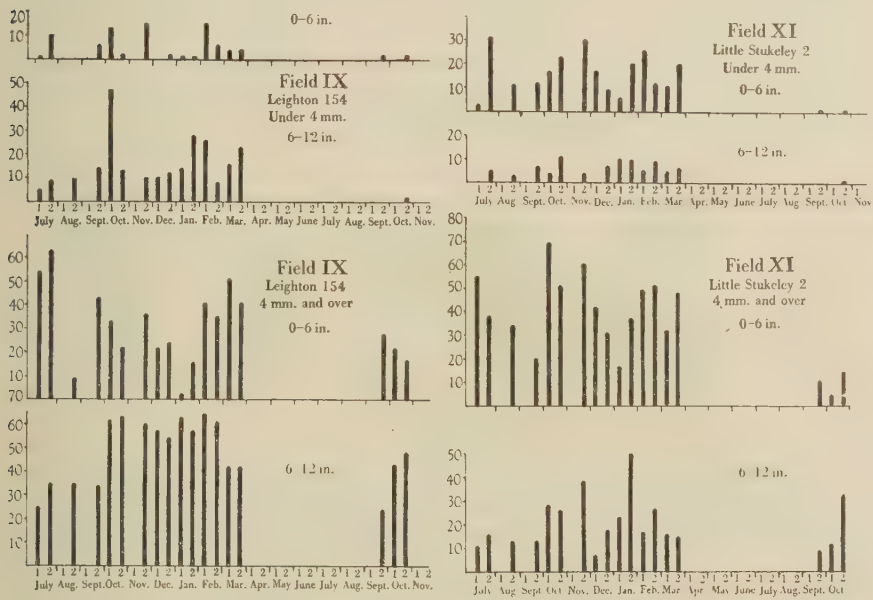

Fig. 3. Depth distribution of wireworms of fields IX and XI.

We have chosen again fields IX and XI to illustrate the distribution of wireworms between the two layers throughout the investigation. Although the numbers in the two layers fluctuate widely on different occasions, Fig. 3 shows that in field IX there are consistently lower numbers and in field XI consistently higher numbers in the surface layer. It may be significant that field IX was ploughed deeply and then cross-ploughed, leaving most of the turf lying on the surface, while field XI was shallow ploughed and lightly cultivated at intervals.

A feature of the populations recorded in the 0–6 in. layer is an apparent decline in numbers during December and January. This occurred to some extent in all fields but was especially marked in fields IX (Fig. 3) and XII. In most cases there is no corresponding decline in the 6–12 in. layer and in fields VII, IX, XI (Fig. 3) and XII the decline in the 0–6 in. layer is accompanied by a rise in numbers in the 6–12 in. layer. This suggests that the dip in total population that was observed to some extent in all fields and was particularly marked in fields VII, IX, XI and XII, is due to a migration of wireworms from the surface layer to points below the depth of sampling. The distribution of wireworms between the two layers is not altered greatly after cropping.

(*e*) *Subsequent history and crop results*

In November 1945 the six fields were sampled again to find out how much the population had declined during the second year of cropping. Table 2 sets out the results of these tests together with a record of the crops grown. It is evident that after two crops the populations have been reduced to a much lower level. In most cases the initial differences between the fields have disappeared and the populations tend to be grouped around the 500,000 per acre level.

Table 2.

Field	Original population (total)	First crop after grass	Population after first crop (total)	Second crop after grass	Population after second crop (total)
VII	4,925,000	Wheat	3,325,000	Wheat	800,000
VIII	3,225,000	Peas	850,000	Potatoes	725,000
IX	2,125,000	Peas	1,350,000	Wheat	500,000
X	4,150,000	Barley	1,225,000	Barley	550,000
XI	1,725,000	Peas	525,000	Potatoes	100,000
XII	2,625,000	Barley	1,000,000	Barley	425,000

Discussion

The results we have obtained on the ploughed fields may be interpreted in two ways. If they are treated as independent data without reference to the grass 'controls', we should conclude that summer and autumn ploughing have little effect in reducing the wireworm population before the following spring. The main reduction during the first year of cropping occurs between April and September. It is due partly to the failure of a new generation of larvae to appear and to mortality amongst the older generations.

If the results we have obtained are examined against the background of the grass 'controls', a different picture is obtained. Fig. 4 shows curves made up of the monthly wireworm collections from the six 'controls' and the six ploughed fields. The difference between the two curves should indicate the effects that can be attributed to the change from grass to a ploughed and cropped condition. Although it is true that no significant fall in population occurs between July 1943 and March 1944 on the ploughed fields, the apparent rise in populations observed in the grass

fields does not occur. Therefore, a net decline of considerable magnitude has taken place. By the same type of analysis it can be seen that, because a decline in population occurs normally on grass fields between the months of April and July, the effect which can be attributed to cropping between April and September is less than the figures from the ploughed fields alone would indicate. This comparison leads us to the conclusion that ploughing in summer and cropping in the following winter or spring soon reduces the population far below the figure it would have reached had the field remained in grass. Moreover, the drop occurring on the grass fields between

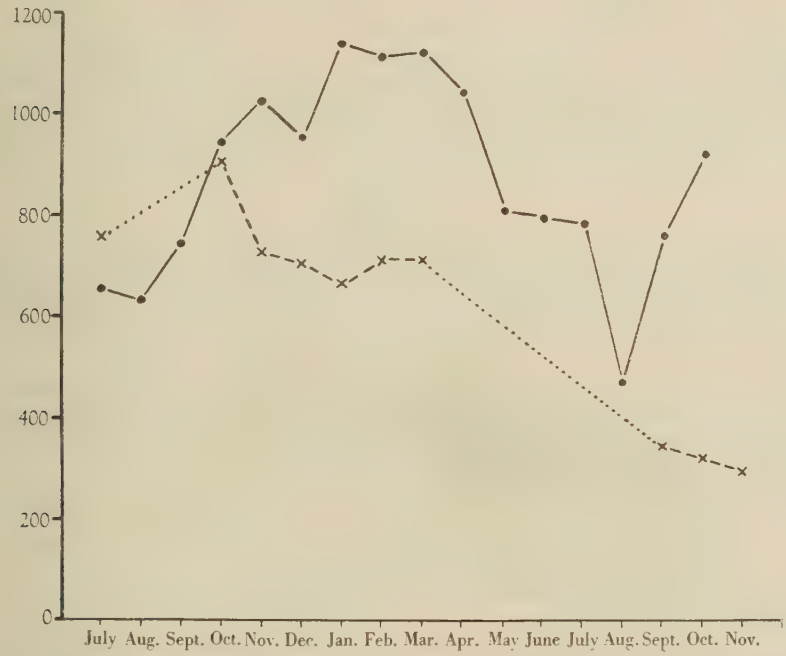

Fig. 4. Monthly averages of wireworms collected from grass and ploughed fields.
–•–•– Grass. –×–×– Ploughed.

April and July is bigger than on cropped fields and the late summer populations of the two types of field are not widely different. The most significant feature of the ploughed fields is that there is no recovery from the low populations observed after the end of the harvest. In other words, the permanent reduction of the population which follows ploughing and cropping comes about, in the long run, mainly by preventing the influx of the new generation of larvae into the population.

The final interpretation of these results must await a satisfactory interpretation of the anomalous populations observed in the grass fields. This question has been discussed fully in the preceding paper. In the meantime, the evidence that the

biggest absolute decline in population occurs during the spring and summer may give some guidance to farmers in selecting dates for ploughing that will bring about the largest reduction in the wireworm population with the least wastage of grassland. Our results indicate that there is little to choose in this respect between ploughing in summer or autumn and ploughing in late winter for a spring crop. In the next paper, we shall present data on the effect of spring ploughing followed by a summer fallow. These data will throw additional light on the problem of selecting the best time of year for breaking up wireworm-infested grassland.

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WIREWORM POPULATIONS IN RELATION TO CROP PRODUCTION

IV. POPULATION CHANGES DURING A BARE FALLOW

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(With 3 Text-figures)

Changes in wireworm populations during a bare fallow were studied on twenty-three fields in 1943 and 1944. In every case populations were much reduced, sometimes to less than 10% of the original level. Wireworms of all sizes were affected, but the larvae less than 5 mm. in length were practically eliminated from the populations.

Evidence is presented that the reduction achieved is greater in fields ploughed in February and March than in fields ploughed in May.

As the populations in the grass 'controls' decline during the same period the entire reduction cannot be attributed to the fallow. The reduction in the bare fallow, however, differs from that in the controls in that it is permanent and continues through the autumn when the counts on the 'controls' are rising.

The practical implications of the results are discussed.

Introduction

The belief that a bare fallow greatly reduces the number of wireworms in a field is very widely held amongst farmers. The grounds for this belief are simply that it is difficult to imagine wireworms surviving in soil that is thoroughly dried and in which most of the grass and weeds that form their food have been destroyed. Many farmers also claim that little wireworm damage occurs after a bare fallow.

No data of a scientific character exist either upon the effect of fallowing on wireworm populations or on crop results after fallowing. It is obvious that there is a need for precise data on this matter and that such data might well find an application in farming practice.

Methods which make it possible to carry out precise studies on wireworm populations have only been developed in recent years (Salt & Hollick, 1944; Cockbill *et al.* 1945). One such method has been used by us for routine advisory sampling of wireworms since 1942. It has also been used in investigations described in the two preceding papers of this series on populations in permanent pastures and in newly ploughed grassland (Ross *et al.* 1947; Cockbill *et al.* 1947). These investigations and the present one on fallowing were undertaken as a single project aiming to show the effects of certain established methods of cultivation on the wireworm population in ploughed-up grassland. In this project, the grass fields sampled throughout 1943–4 are regarded as 'controls'.

In the preceding paper on population changes following summer ploughing (loc.

cit.), we reported that a marked decline in population occurred only in the spring and summer of the following year. This coincides with the period of declining and minimal populations observed on the 'controls' from April to July. These facts suggest that population changes of a most interesting kind occur at this time of year in all types of land. Since summer fallows cover all or part of this period their effects should be of special interest.

The opportunity to investigate the effect of fallowing arose mainly in Essex where it is a common practice to expose rough grass land to a bare fallow after ploughing. Elsewhere in Eastern England bare fallows are rare, most farmers preferring to use the grass for pasture or hay in spring and summer, and ploughing in July at the earliest for winter or spring cropping.

Results

The investigation was begun on nine fields in 1943 and continued on fourteen other fields in 1944. The fields were sampled in the usual way (twenty cores, 4 in. in diameter) and the wireworms collected by the washing and flotation process previously described (Cockbill *et al.* 1945). In 1943 the samples were taken to a depth of 6 in. before ploughing and 8–9 in. during the fallow. In 1944, all samples were taken to a depth of 12 in. and the 0–6 and 6–12 in. fractions were examined separately.

1943 *series*

Of the nine fields studied in 1943, seven (nos. 1–7) were in Essex and two (nos. 8–9) in Huntingdonshire. Nos. 1–4 and 8–9 were on medium loam over clay and nos. 5–7 were on marsh silt. The fields were sampled at least once before the end of March and subsequently three or four times during the summer and autumn. Nos. 1–4 were already ploughed when first sampled but all the others were in grass.

Table 1. *Total wireworms in* 1000*'s per acre at successive samplings*

Field	Date of ploughing	1st sampling Feb./March	2nd sampling July/Aug.	3rd sampling October	4th sampling November	5th sampling Dec./Jan.
1	Feb.	1,700	700	300	50	175
2	Feb.	2,375	725	800	375	525
3	Feb.	850	250	350	50	75
4	Feb.	4,800	1,125	575	250	400
5	May	4,275	900	575	225	350
6	May	2,625	450	825	1,125	625
7	May	2,775	800	750	800	825
8	May	4,100	1,400	—	575	350
9	June	2,150	775	—	375	75
Total		25,650	7,125	4,175	3,825	3,400
Average		2,850	792	—	425	377

Table 1 shows the resulting populations at successive samplings. It is apparent that a progressive decline in population occurred in all nine fields. The major part of the reduction had already taken place by August. Over the whole period the populations fell to less than 13% of their original level and in several fields to less

than 8%. This decline greatly exceeds that following cropping when populations normally fall to 50% of their original level. Even after two years of cropping, the average reduction is only 33% (Cockbill *et al.* 1945).

When the wireworms are arranged in order of lengths, a size distribution is obtained. This is shown in Table 2 for fields 1–7 taken together for four sampling periods.

TABLE 2. *Size distribution in mm. Seven fallow fields together*

Period	<4	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Totals
February	173	124	84	53	80	70	28	44	33	35	19	17	20	10	7	5	0	7	809
July	20	16	16	12	13	10	4	14	6	10	4	5	8	8	4	0	1	2	153
October	8	12	14	9	15	17	5	5	13	8	6	6	3	5	2	2	0	0	130
November	0	3	14	15	9	8	7	8	10	4	9	8	8	2	0	2	2	0	109

It is seen that at the first sampling, wireworms of length less than 5 mm. formed 37% of the total. This is a normal figure for the percentage of wireworms of this size in old grassland. At the last sampling, these small forms had all but disappeared, comprising less than 3% of the much-reduced population. The general decline in population and alteration in the numbers in the different size groups is shown in the histograms (Fig. 1) which are based on Table 2. It is evident that although the effect of the fallow is greatest on the smallest forms, actually wireworms of all sizes are affected, although the largest may have pupated.

1944 *series*

The 1944 fields can be described most conveniently in two groups. The Latchingdon group consisted of six fields (nos. 10–15), five in Essex and the remaining one in Bedfordshire. The soils were classified as medium loam over clay on field 10, heavy loam on field 11, medium valley loam on fields 12–13 and medium silt on fields 14 and 15. All these fields were ploughed early in the year (March or earlier). The Nazeing group consisted of eight fields (nos. 16–23) on a single farm at Nazeing in Essex. The soil was classified as clay with flints. These fields were not broken until May when they were cultivated and the turf torn up. Further cultivations took place at frequent intervals and in due course all were ploughed.

Figs. 2 and 3 show the wireworm counts of four fields from each group at successive samplings. Also shown are graphs of the running means of four consecutive counts, the first point being the average of counts 1–4, the second of counts 2–5, etc. This method of analysis proved very useful in the preceding papers in showing up trends in population. The fields selected for the Figures are typical of the groups and have been chosen to show examples with high, low and intermediate populations.

The population changes seen in the Figures obviously bear out the results obtained in 1943, showing a marked fall in population during the fallow period in all the fields examined. This is a gradual process in every case and only occasionally is there any suggestion of an upward trend.

The fields in the Latchingdon group were all ploughed by March and fell to populations below 500,000 per acre. The fields in the Nazeing group were broken up in May and did not show such a marked decline, especially those which maintained higher populations at the beginning. Table 3 shows the average populations at the beginning and end of the experiment for both groups. The final counts on the Latchingdon group show that all but 22% of the wireworms have disappeared during the fallow, whereas the corresponding figure for the Nazeing group is 36%.

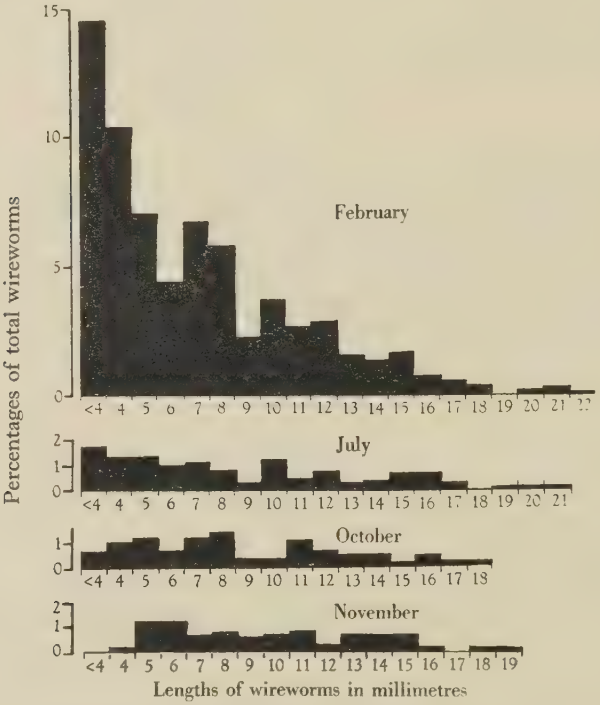

Fig. 1. Size distribution of wireworms from seven fallows (fields 1–7, 1943 series) taken together at four successive samplings, expressed as a percentage of the total number of wireworms found.

Table 3

	Average populations of	
	First 4 samplings	Last 4 samplings
Latchingdon group (6 fields)	2,275,000	500,000
Nazeing group (8 fields)	2,950,000	1,075,000

The smaller larvae had almost completely disappeared during the fallow in the Latchingdon group. In the Nazeing group, more of the small larvae survived the

fallow but their numbers were also greatly reduced. There was no trace of an influx of newly hatched individuals during the summer months on any of the fields.

In the Latchingdon group the proportions of wireworms collected from the two depth fractions were variable. In field 11, the numbers were about equal in the two layers, whereas in fields 10 and 12, the upper layer contained up to twice as

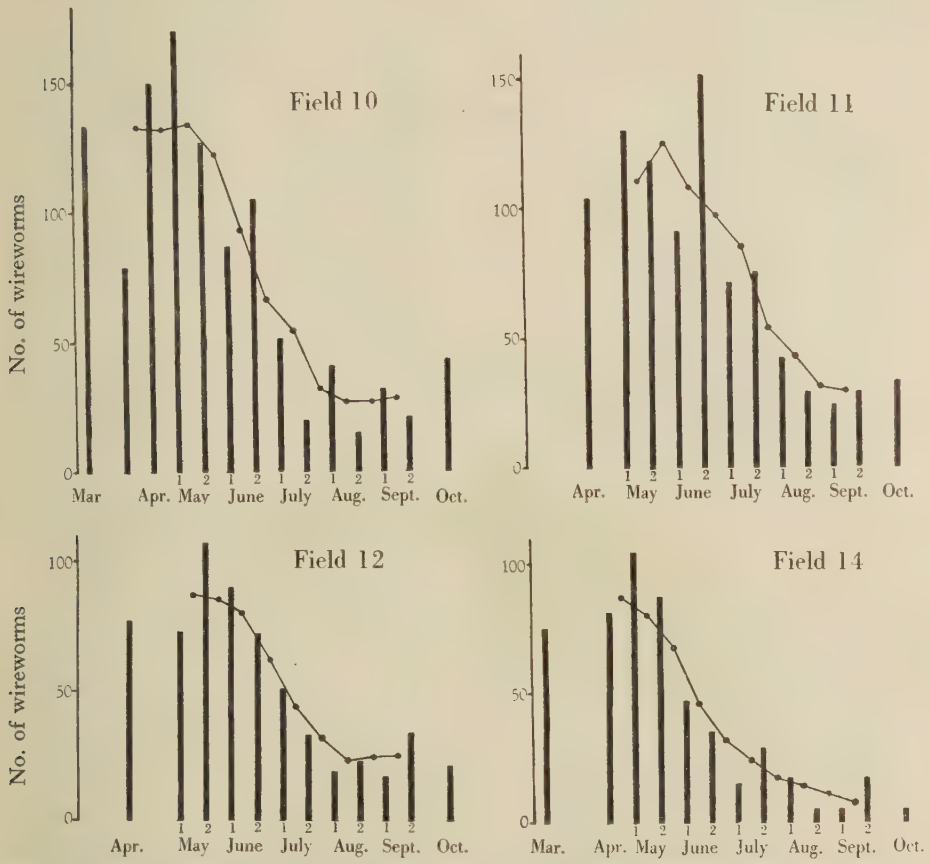

Fig. 2. Number of wireworms (histograms) found at successive samplings and running means of four consecutive samplings (graphs) on typical fields of the Latchingdon group.

many. In all these fields the decline in population was evident in both layers and the decline occurred at approximately the same rate.

In the Nazeing group, the numbers of wireworms in the lower fraction remained relatively constant, usually between 250,000 and 500,000 per acre. The main decline occurred amongst the wireworms in the 0–6 in. layer.

In all except two of the counts (fields 11 and 14 in July), fewer wireworms were

recorded in the 6–12 in. layer. There was no evidence that wireworms left the upper and probably drier regions of the soil for the deeper layers below.

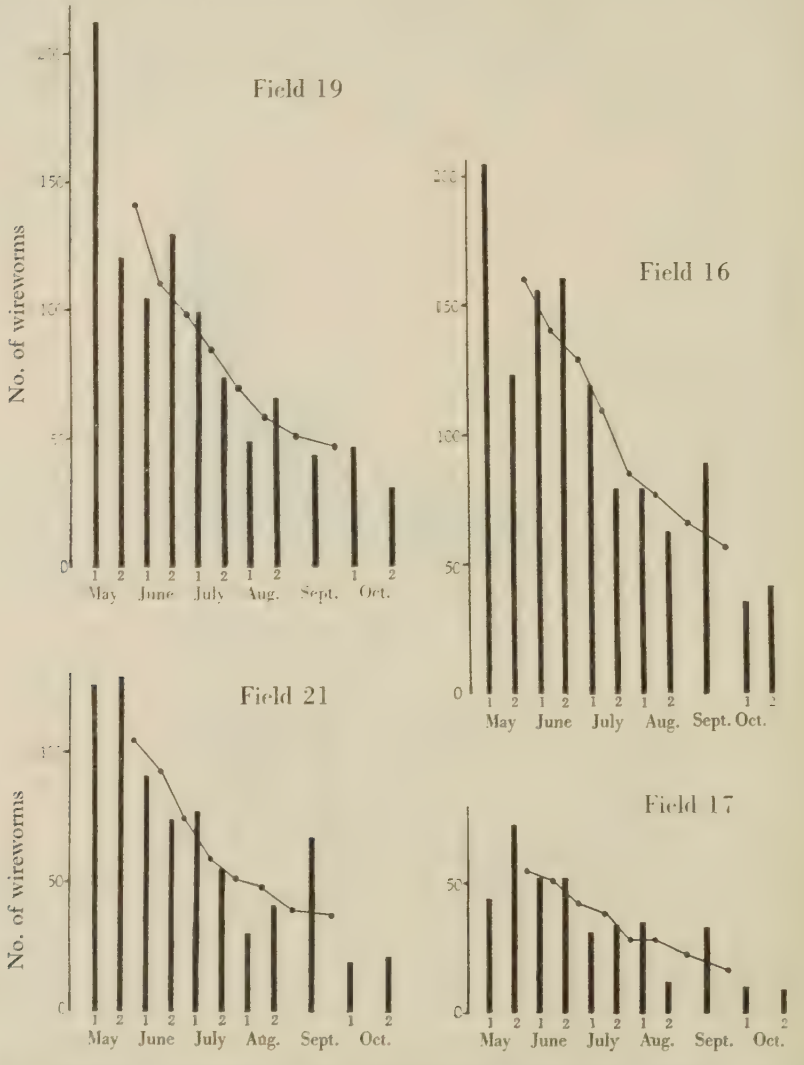

Fig. 3. Number of wireworms (histograms) found at successive samplings and running means of four consecutive samplings (graphs) on typical fields of the Nazeing group.

Crop results

The subsequent history of the fields was followed in order to test the belief that wireworm failures do not occur after a fallow. Obviously there are too few fields

to enable any conclusions to be drawn but they showed certain points of some interest.

In the 1943 series, all the fields in Essex grew good crops (nos. 1–7), five growing wheat and the remaining two beans. On the other hand, the two Huntingdonshire fields (nos. 8 and 9) were both failures. Field 8 grew potatoes, sugar beet and kale, all of which were retarded by drought. The potatoes and beet were severely attacked by wireworms which were a secondary if not a primary cause of failure. Field 9 was put into wheat in the autumn of 1943 and, in spite of the low population recorded at the final sampling (375,000 per acre), it failed completely from wireworm attack.

In the 1944 series, cereals were grown on most of the fields and no failures due primarily to wireworms were reported. On fields 16 and 21 of the Nazeing group, rabbits retarded the growth of the wheat by close cropping throughout the winter. The crops were badly damaged by wireworms in the spring; in fact these fields might have been regarded as wireworm failures if the rabbit damage had not been observed first.

As all the fields studied in this investigation had high populations at the beginning, it is likely that many of them would have failed from wireworm attack had they been ploughed and sown directly. The fact that only one of the twenty-three fields could be described as a true wireworm failure is some evidence that a bare fallow reduces the danger of wireworm attack. Nevertheless, the fact that a field containing only 375,000 wireworms per acre can fail from wireworm attack suggests that under certain conditions, even very low populations may cause serious damage. This question will be considered again in a later paper.

Strip-ploughing experiments

Attempts were made to test the effect of fallows of different lengths on the wireworm populations by ploughing up different strips of single fields at different times. This experiment might have provided more conclusive evidence on the points under discussion, but it proved extremely difficult to carry out in practice. In 1944, on two fields in Bedfordshire which were ploughed in May, strips were left to be ploughed later in August. In these cases, however, the strips were only a few yards wide and no differences in populations or crop results could be detected. In 1945, an 8-acre field in west Suffolk was divided into four plots which were ploughed at different times throughout the spring and summer. Wireworm counts were taken at intervals to follow changes in the population. Unfortunately, plots 1 and 2 were planted with cabbages in June, in spite of the arrangement to leave the whole field fallow, so that these plots were not in fact left fallow and the cabbages made it difficult to rely on the random character of the samples. The results of the experiment are given in Table 4.

The results indicate that the reduction in population is greater on the earlier ploughed plots and especially that there is a marked difference between the plots ploughed before and after July. In fact plots 1, 2 and 3 behaved like the bare fallows

described above and plot 4 like the fields ploughed in July described in the preceding paper (Cockbill *et al.* 1947). However, it would be premature to base any conclusions on this particular experiment. It is reported here mainly to show the difficulty of carrying out even the simplest experiment on wireworm work as long as land and other facilities are not strictly under the control of the experimenter.

Table 4. *Wireworm populations per acre on four plots on a field in west Suffolk ploughed at different times from April to August* 1945

Counts made in	Plot 1 Ploughed in early April	Plot 2 Ploughed in mid-May	Plot 3 Ploughed in early June	Plot 4 Ploughed in mid-August
March 1945	2,800,000 (plot ploughed)	2,500,000	1,800,000	2,600,000
May	1,700,000	2,000,000 (ploughed)	2,000,000	1,700,000
June	2,100,000	1,700,000 (cabbages planted)	1,800,000 (ploughed)	1,900,000
August	900,000	900,000	2,300,000	2,200,000 (ploughed)
October	400,000	600,000	1,200,000	1,400,000
November	1,000,000	800,000	1,000,000	2,100,000
January 1946	500,000	900,000	800,000	1,600,000

Discussion

The evidence from all twenty-three fields investigated clearly confirms the view held by farmers that a bare fallow leads to a big reduction in the wireworm population. This reduction usually exceeded that experienced after cropping, even for two successive years. The figures indicate clearly that the reduction was greater for the earlier ploughed fields of the Latchingdon group than for the later ploughed fields of the Nazeing group in 1944.

It should be remembered, however, that in the period from April to July, the populations on grass fields undergo a big decline. On the 'controls' for these investigations (Ross *et al.* 1947) the populations declined on the average to about 50% of the maximum level recorded in January–March. Therefore, we cannot attribute the entire reduction that occurred in the fallowed fields to the special conditions operating in those fields. A large part of the reduction would have occurred even if the fields had remained in grass. Nevertheless, fallowing intensifies this process during these months. Its full effect becomes apparent only in the months after July, when the grass 'controls' showed an influx of new individuals and rising counts throughout the autumn and winter. During this period, the fallowed fields continue to decline in population and, in the long run, it is this part of the process which leads to the permanent reduction of the population.

The actual cause of the death or disappearance of the wireworms is uncertain but it is clearly a progressive and not a sudden occurrence. In all probability it is due

to the operation of several factors, some of which may be acting with maximum effect at different times. Possibly the reduction of food during the period of maximum activity and growth in the spring has some effect. Later on the drying out of the soil may increase the abrasion of the cuticle and so cause mortality through water loss. Wigglesworth (1945) has shown that *Agriotes* larvae are particularly susceptible to water loss from this cause. Finally, the lack of cover during the summer months must lead to a high mortality amongst eggs and newly hatched larvae.

The practical value of these results is fairly obvious. Clearly, great reductions in wireworm population can be achieved by exposing broken-up grassland to a bare fallow. In order to achieve the greatest reduction in the population in a fallow, ploughing of the old grassland should take place in February or March and not later than April. In breaking up old grassland during the war it has seldom been permissible to subject the land to a bare fallow as a hay crop was usually taken before the land was broken. While this may have been a necessity, in the writers' experience, this procedure all too often resulted in a failure of the first crop (wheat or oats) from wireworm and sometimes of the second crop as well. In many such cases it would have been better to have taken a full fallow in the first season. Perhaps it may be possible in future to incorporate fallowing into farming practice where it is advisable to reduce wireworm populations before sowing the first crop on newly ploughed grassland.

The authors wish to repeat the acknowledgements made in previous papers to Mr F. R. Petherbridge, M.A., in whose department the work was carried on, for his help and encouragement, and to the assistant staff for their unfailing co-operation. Acknowledgement is due to Mr V. E. Henderson, formerly a member of the Department, who originally suggested and initiated the investigation in Essex. Thanks are also due to those members of the Essex, Huntingdon and West Suffolk W.A.E.C. staffs who suggested the fields for these tests and without whose assistance the work would have been impossible.

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ANNONA SPECIES AS INSECTICIDES

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(With 2 Text-figures)

By extraction and precipitation from several solvents the toxic principle present in *Annona reticulata* and *A. squamosa* seeds and roots has been concentrated up to one hundred-fold. A preliminary chemical examination of this concentrate is described, leading to the conclusion that the toxicity is due to a glyceride or glycerides of a hydroxylated unsaturated acid or acids of high molecular weight.

These extracts have been examined for contact and stomach poison and ovicidal properties in a variety of media. When used as a contact insecticide against *Aphis fabae*, *Macrosiphoniella sanborni* and *Macrosiphum solanifolii*, the concentrate exhibited a toxicity of the same order as that of rotenone, but against *Oryzaephilus surinamensis* the toxicity was considerably less. As a stomach poison the ether extract was both toxic and repellent to *Plutella maculipennis* larvae, but was neither toxic nor repellent to *Diataraxia oleracea* larvae. Ovicidal tests against the eggs of *Plutella maculipennis* and of *Ephestia kühniella* were inconclusive. The potency of this concentrate is therefore of a limited nature and although of roughly the same order as that of rotenone to certain aphides, it has neither the intensity of effect nor the range of insecticidal action of that compound.

Introduction

The genus *Annona* consists of about ninety species of trees and shrubs distributed mainly in tropical America, but a few are natives of tropical Asia and Africa. It is of some economic importance; edible fruits such as cherimoya, custard-apple, sour sop, sweet sop are derived from certain species; various parts of the plants of several of the species have some repute medicinally, also for stupefying fish, and for insecticidal purposes. McIndoo & Sievers (1924) mention five species, the seeds of which are reputed to have insecticidal action: *A. cherimola*, *A. glabra*, *A. reticulata*, *A. spinescens* and *A. squamosa*. In a report issued by the Department of Agriculture, Mysore (1938) the insecticidal effect of alcoholic extracts of parts of *A. reticulata* upon the scale insect, *Lecanium viride* Green, is described. No information is given as to the method of testing employed, but it was claimed that to this insect the alcoholic extract of the seed of *Annona reticulata* was more toxic than *Derris elliptica*. In view of these results Tattersfield & Potter (1940) carried out laboratory trials with several species, *Annona muricata*, *A. palustris*, *A. reticulata*, and *A. squamosa*. All of these showed contact insecticidal properties to the aphides *Aphis fabae* and *Macrosiphoniella sanborni*. *Annona reticulata* was the most toxic; seed, root, leaf, and to a lesser extent the stem, of this species possessing contact insecticidal properties.

The chemistry of plants of the genus *Annona* has been little investigated. Callan & Tutin (1911) isolated from the leaves of *A. muricata* a steam-volatile essential oil, an amorphous alkaloid, several fatty acids, myricyl alcohol, sitosterol, and a compound 'anonol',* later shown by Power & Salway (1913) to be a phytosterol glucoside.

Trimurti (1924) isolated an alkaloid from the leaves of *A. squamosa* but in too small a quantity to investigate. Santos (1930, 1932) and Reyes & Santos (1931) isolated an alkaloid, anonaine,* from the bark of *A. reticulata* and *A. squamosa* which they characterized. Barger & Weitnauer (1939) established the constitution of anonaine as 5:6-methylenedioxyaporphine and synthesized an optically inactive form of the base. Meyer (1941) has likewise isolated two alkaloids of the aporphine group from the bark of *A. muricata*. Many analyses of the seed oils of these species of differing geographical origin have been published with a view to their economic utilization as food stuffs.

For the purposes of this investigation, to effect a concentration and if possible identify the active principle or principles responsible for the insecticidal properties of *Annona*, the seeds of *A. reticulata* from Ceylon were initially selected. Ether extraction of the ground air-dried seeds gave 32·5% of an orange-coloured oil which was appreciably toxic to aphides (see Table 2). As it was realized that the seed oil itself was unlikely to be responsible for the toxicity and following the observation of Ghanekar & Ayyar (1927), who showed that the seed oil of *A. squamosa* contained 1·2% of a petroleum ether-insoluble resin, the *A. reticulata* extract was treated with five volumes of petroleum ether (b.p. 40–60° C.) and kept at 0° C. A brown cheesy mass separated and was recovered by decantation (6·3% on the oil). Biological trials (see Table 2) showed that this fraction contained all the toxic material, thus representing a sixteen-fold concentration. Subsequent variation of conditions showed that less extraneous material was precipitated by conducting the separation at room temperature. The resinous concentrate so obtained (2·55% on the oil, 0·8% on the seeds; i.e. a 120-fold concentration) was very highly toxic to aphides (see Table 3), approaching that of rotenone itself. Similar petroleum ether-insoluble resins have been prepared from the seeds and other parts of several species of *Annona* and are summarized in Table 1 and their insecticidal properties in Table 4.

TABLE 1. *Analytical data of two species of* Annona

				Ether-soluble extract		Petroleum ether-insoluble extract	
Species	Ref. no.	Part of plant	Source	% on seeds or root	Equivalent weight by saponification	% on seeds or root	Equivalent weight by saponification
A. reticulata	Y10	Seeds	Ceylon	32·5	297	0·8	506
	Y12	Root	Ceylon	3·7	—	1·5	—
A. squamosa	Y13	Seeds	Ceylon	27·3	306	1·7	568
	Y21	Seeds	India	26·0	305	2·0	597

* Strictly speaking, anonol and anonaine should become annonol and annonaine respectively as the spelling *Anona* is no longer accepted.

Chemical investigation of the toxic concentrate from *A. reticulata* seeds

Element tests showed the presence of carbon, hydrogen and oxygen only. The absence of nitrogen disposes of the possibility that the toxicity is due to the alkaloidal constituents of *Annona*. Tests for sterols and rotenoids were negative. The acrolein test for glycerol was positive suggesting the presence of glycerides, which was confirmed by saponification.

Analytical data on concentrate: equivalent weight by saponification, 506; iodine number, 29·3; carbon, 71·5%; hydrogen, 10·5%; unsaponifiable fraction, 3·9%; insoluble acids, 96·2%.

Saponification. Hydrolysis with alcoholic potash, dilution with water and ether extraction gave the unsaponifiable fraction. Acidification of the aqueous layer gave the free insoluble acids as a dark red viscous resin which did not crystallize.

Unsaponifiable fraction. This had an aromatic smell, but was non-toxic and not therefore further examined. The toxicity of the concentrate is therefore due to the glyceride fraction.

Analytical data on the free insoluble acids: carbon, 71·4%; hydrogen, 10·65%. A formula $C_{32}H_{58}O_6$ (requires carbon, 71·4%; hydrogen, 10·85%; molecular weight, 539) fits the data, but as the material is not thought to be homogeneous no great stress is laid on the exact number of carbon and hydrogen atoms. Nevertheless the figures serve to indicate the highly oxygenated acidic nature of the product. The iodine number is 28·0, corresponding to 0·60 double bonds on a molecular weight of 539.

A portion of the acid was acetylated by refluxing with acetic anhydride for 2 hr. The product was boiled successively with water until the aqueous layer was neutral and then dried to constant weight at 100° C. This gave an acetyl value of 196, corresponding to 1·9 hydroxyl groups on a molecular weight of 539.

It has not been possible to continue the chemical investigation further owing to the departure of one of the authors (S. H. H.) and the pressure of other work. Nevertheless, it is concluded that the toxic principle of *Annona* is a glyceride of one or more hydroxylated acids of unusually high molecular weight.

Biological investigation

Contact insecticidal properties

The trials listed in Table 2 were carried out using the apparatus and technique of Tattersfield & Morris (1924), (Tattersfield, 1934); whereas those listed in Tables 3 and 4 were carried out using the apparatus and technique of Potter (1941). The slowness of paralytic action of *Annona* extracts observed by Tattersfield & Potter (1940) has been confirmed. For that reason the insects were not examined until 40–48 hr. after spraying.

TABLE 2. *Comparison of contact insecticidal properties of two fractions of extracts of the seed of* Annona reticulata

Test insect: *A. fabae* Scop. (a wild colony). Date: 23 July 1940. Medium: 0·5 % w/v saponin—10 % v/v alcohol.

	Concentration (mg./l.)	Mortality	Corrected % kill
A. reticulata seeds Y 10			
Ether-soluble extract	1033	35/45	75
	775	27/44	58
	517	17/44	31
	258	8/45	2
Equation to the probit line: x = log (conc. in mg./l.)	$Y = -5{\cdot}06 + 3{\cdot}55x$, L.D.$_{\cdot 50}$ = 676 mg./l.		
Petroleum ether-soluble extract	1103	12/44	18
Petroleum ether-insoluble extract (6·3 % on the oil)	250	47/48	98
	125	37/41	89
	62·5	34/43	76
	31·2	11/45	15
Control	0	5/44	—
Equation to the probit line: x = log (conc. in mg./l.)	$Y = -0{\cdot}73 + 3{\cdot}38x$, L.D.$_{\cdot 50}$ = 50·1 mg./l.		

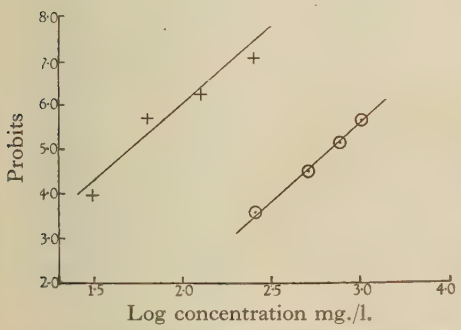

Fig. 1. Date 23 July 1940. Comparison of the relative toxicity to *Aphis fabae* Scop. of an ether-soluble extract and a petroleum ether-insoluble extract of *Annona reticulata* seed Y 10.

⊙ = Ether-soluble extract.
\+ = Petroleum ether-insoluble extract.

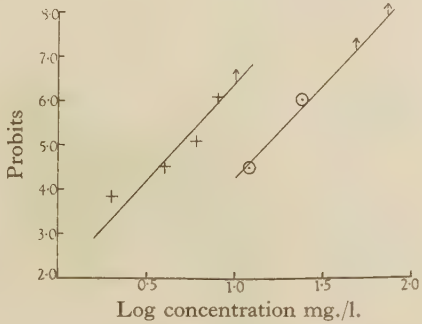

Fig. 2. Date 22 July 1941. Comparison of the relative toxicity to *Macrosiphoniella sanborni* Gill. of a petroleum ether-insoluble extract of *Annona reticulata* seed Y 10 and rotenone.

⊙ = *Annona reticulata*. \+ = Rotenone.
↑ = Dose at which 100 % kill occurred.

Table 2 and Fig. 1 show that the ether-soluble extract of *A. reticulata* was toxic to *Aphis fabae* Scop. (black-bean aphis). The petroleum ether-insoluble extract derived from it was considerably more toxic than the ether-soluble extract and appeared to contain practically all the toxic material, since the petroleum ether-soluble fraction had an insecticidal value very little if any different from the controls. This was confirmed by later experiments the data of which are shown in Table 3(ii), in which *Macrosiphoniella sanborni* Gill. (chrysanthemum aphis) was used.

TABLE 3. *Comparison of contact insecticidal properties of various fractions of extracts of* Annona reticulata *and rotenone*

	Concentration (mg./l.)	Mortality	Corrected % kill
(i) Test insect: *M. sanborni.* Date: 22 July 1941. Medium: 0·5 % w/v saponin—10 % v/v alcohol.			
A. reticulata seed Y 10	96	28/28	100
Petroleum ether-insoluble extract	72	29/29	100
	48	27/27	100
	24	25/29	85
	12	7/20	31
	0	2/31	—
Equation to the probit line: x = log (conc. in mg./l.)	$Y = 0{\cdot}08 + 4{\cdot}20x$, L.D.$_{50}$ = 15 mg./l.		
Rotenone	10	30/30	100
	8	26/30	86
	6	17/30	54
	4	7/20	31
	2	5/30	12
	0	2/31	—
Equation to the probit line: x = log (conc. in mg./l.)	$Y = 1{\cdot}93 + 4{\cdot}52x$, L.D.$_{50}$ = 4·8 mg./l.		
(ii) Test insect: *M. sanborni.* Date: 30 October 1941. Medium: 0·5 % w/v saponin—10 % v/v alcohol.			
A. reticulata seed Y 10	500	29/31	94
Ether-soluble extract	375	26/32	81
	250	9/30	30
	125	1/30	3
	0	0/30	—
Equation to the probit line: x = log (conc. in mg./l.)	$Y = -10{\cdot}12 + 6{\cdot}17x$, L.D.$_{50}$ = 280 mg./l.		
A. reticulata seed Y 10	8	15/29	52
Petroleum ether-insoluble extract	6	23/28	82
	4	10/29	34
	2	7/30	23
	0	0/30	—
Equation to the probit line: x = log (conc. in mg./l.) + 1	$Y = 1{\cdot}76 + 1{\cdot}92x$, L.D.$_{50}$ = 4·9 mg./l.		
A. reticulata seed Y 10	250	30/30	100
Free insoluble *acids* from petroleum ether-insoluble extract	50	26/29	90
	10	16/30	53
	0	0/30	—
Equation to the probit line: x = log (conc. in mg./l.)	$Y = 3{\cdot}23 + 1{\cdot}84x$, L.D.$_{50}$ = 9·1 mg./l.		
Rotenone	4	31/31	100
	3	28/30	93
	2	22/30	73
	1·5	23/30	77
	1·0	14/29	48
	0	0/30	—
Equation to the probit line: x = log (conc. in mg./l.) + 1	$Y = 1{\cdot}61 + 3{\cdot}33x$, L.D.$_{50}$ = 1·0 mg./l.		
(iii) Test insect: *M. sanborni.* Date: 11 August 1941. Medium: 0·1 % w/v sulphonated lorol—10 % v/v alcohol.			
A. reticulata seed Y 10	20	28/31	88
Petroleum ether-insoluble extract	10	21/30	65
	4	12/30	29
	0	3/20	—
Equation to the probit line: x = log (conc. in mg./l.)	$Y = 2{\cdot}96 + 2{\cdot}46x$, L.D.$_{50}$ = 6·8 mg./l.		
Rotenone	5	30/30	100
	1	17/30	49
	0·5	8/30	14
	0	3/20	—
Equation to the probit line: x = log (conc. in mg./l.) + 2	$Y = -2{\cdot}64 + 3{\cdot}83x$, L.D.$_{50}$ = 1·0 mg./l.		

Table 4

(i) Comparison of the toxicity of the petroleum ether-insoluble extracts of the seed of *A. reticulata* and *squamosa*.

(ii) Effect of media on toxicities of petroleum ether-insoluble extracts of root of *A. reticulata*.

(iii) Comparison of the toxicities of petroleum ether-insoluble extracts of the root of *A. reticulata* and rotenone to *O. surinamensis*.

	Concentration (mg./l.)	Mortality	Corrected % kill
(i) Test insect: *M. sanborni*. Date: 19 December 1941. Medium: 0·1 % sulphonated lorol—10 % v/v acetone.			
A. reticulata seed Y 10	20	21/30	70
Petroleum ether-insoluble extract	15	19/30	63
	10	10/30	33
	5	7/30	23
	0	0/20	—
Equation to the probit line: x = log (conc. in mg./l.)	$Y = 2{\cdot}61 + 2{\cdot}21x$, L.D.$_{50}$ = 12 mg./l.		
A. squamosa seed Y 13	250	30/30	100
Petroleum ether-insoluble extract	50	25/26	96
	10	18/30	60
	0	0/20	—
Equation to the probit line: x = log (conc. in mg./l.)	$Y = 3{\cdot}07 + 2{\cdot}18x$, L.D.$_{50}$ (outside range of kill) = 7·8 mg./l.		
A. squamosa seed Y 21	250	31/31	100
Petroleum ether-insoluble extract	50	29/30	97
	10	25/29	86
	0	0/20	—
Equation to the probit line: x = log (conc. in mg./l.)	$Y = 4{\cdot}79 + 1{\cdot}28x$, L.D.$_{50}$ (outside range of kill) = 1·5 mg./l.		
Rotenone	2·0	27/30	90
	1·5	27/31	87
	1·0	20/28	71
	0·5	15/38	39
	0	0/20	—
Equation to the probit line: x = log (conc. in mg./l.) + 1	$Y = 2{\cdot}82 + 2{\cdot}73x$, L.D.$_{50}$ = 0·63 mg./l.		
(ii) Test insect: *Macrosiphum solanifolii*. Date: 7 September 1943. Medium: 0·5 % w/v saponin—10 % v/v alcohol.			
A. reticulata root Y 12	500	46/49	94
Petroleum ether-insoluble extract	250	46/55	83
	100	42/51	81
	50	17/55	26
	25	7/56	6
	0	4/57	—
Equation to the probit line: x = log (conc. in mg./l.)	$Y = 0{\cdot}67 + 2{\cdot}28x$, L.D.$_{50}$ = 79 mg./l.		
Medium: 0·1 % w/v sulphonated lorol—10 % v/v acetone.			
A. reticulata root Y 12	500	50/51	98
Petroleum ether-insoluble extract	250	44/50	88
	100	38/56	67
	50	14/56	23
	25	6/52	10
	0	1/53	—
Equation to the probit line: x = log (conc. in mg./l.)	$Y = 0{\cdot}33 + 2{\cdot}43x$, L.D.$_{50}$ = 83 mg./l.		
(iii) Test insect: *Oryzaephilus surinamensis*. Date: 10 December 1942. Medium: 0·1 % w/v sulphonated lorol—20 % v/v acetone.			
A. reticulata root Y 12	10,000	42/65	64
Petroleum ether-insoluble extract	2,000	11/64	14
	400	2/51	1
	0	3/90	—
Equation to the probit line: x = log (conc. in mg./l.)	$Y = -2{\cdot}77 + 2{\cdot}03x$, L.D.$_{50}$ = 6800 mg./l.		
Rotenone	60	84/106	78
	30	41/87	45
	15	38/82	44
	7·5	30/112	25
	3·75	9/98	6
	0	3/90	—
Equation to the probit line: x = log (conc. in mg./l.)	$Y = 1{\cdot}27 + 1{\cdot}57x$, L.D.$_{50}$ = 24 mg./l.		

Tests, on two different dates, of a petroleum ether-insoluble extract of *Annona reticulata* in saponin and alcohol showed a toxicity to *Macrosiphoniella sanborni* lower than that of rotenone in the same medium. When a sulphonated lorol-acetone medium was used a similar relationship was obtained. The toxicities of rotenone and the petroleum ether-insoluble fraction were, however, of the same order. The relevant data are given in Table 3. The free insoluble acids derived from the extract had a lower toxic effect than the extract itself (Table 3(ii)).

A further series of tests are set out in Table 4. Petroleum ether-insoluble extracts of two samples of *Annona squamosa* in sulphonated lorol and acetone were slightly more toxic to *Macrosiphoniella sanborni* than a similar extract of *Annona reticulata*, but it is doubtful whether the result is significant. There was no apparent difference in toxicity to *Macrosiphum solanifolii* Ash. (potato aphis) between a petroleum ether-insoluble extract of *Annona reticulata* in a saponin-alcohol and a sulphonated lorol-acetone medium. The latter extract was considerably less toxic to *Oryzaephilus surinamensis* L. (saw-toothed grain beetle) than was rotenone in the same medium. It is thus clear that the potency of the petroleum ether-insoluble fraction is of a specific nature and although of roughly the same order as rotenone to certain aphides, has neither the intensity of effect nor the range of insecticidal action of that compound. The relative toxicities of the petroleum ether-insoluble extract of *Annona reticulata* and rotenone are given graphically in Fig. 2.

Stomach poison properties

We are indebted to Mr M. J. Way for carrying out a number of experiments in which the effect of the seed extract was tested out by feeding to *Plutella maculipennis* (diamond-back moth) and *Diataraxia oleracea* (tomato moth).

Material used

7·46 g. ether extract of *Annona reticulata* seeds (in the form of an oil) was diluted to 50 c.c. with ether to form the stock solution.

Methods: Plutella maculipennis.

Disks of $\frac{1}{2}$ in. diameter were cut from cabbage and divided into six batches of seven circles each. These were dipped into ether solutions of the extract as follows:

(1) Stock solution—seven disks.
(2) Stock solution diluted half with ether—seven disks.
(3) Stock solution diluted a quarter with ether—seven disks.
(4) Stock solution diluted 1/8 with ether—seven disks.
(5) Stock solution diluted 1/16 with ether—seven disks.
(6) Control—ether only.

The disks were placed individually in cages with their edges in moist sand. Two early last instar *P. maculipennis* larvae were placed in each cage.

Results obtained by an examination after 3 days were as follows:

Treatment	Effect on larvae*	Extent of feeding
1	6D, 2B, 2S	One or two nibbles only
2	2D, 2B, 3S, 7N	Very little eaten
3	3S, 11N	About 10% eaten
4	14N	About 20% eaten
5	14N	About half eaten
6	14N	All circles completely consumed

* N = not affected, S = slightly affected, B = badly affected, D = dead.

Diataraxia oleracea

Small cabbage plants were dipped in treatments 1–4 of the above and each plant placed in a lamp glass together with twelve early last instar *D. oleracea* larvae.

Examinations made during the following 2 days showed that some of the larvae in treatment 1 were affected probably by the oily film that covered the leaves but after a week the larvae in all the treatments were normal and the plants had been completely consumed except in treatment 1 where phytotoxicity had caused severe damage to the foliage.

Conclusions

An *Annona reticulata* seed extract showed some toxicity to *Plutella maculipennis* larvae at high concentrations. Lower concentrations showed a repellent effect. The *Annona reticulata* extracts were neither toxic nor repellent to larvae of *Diataraxia oleracea.*

Ovicidal properties

In a test (7 January 1943) using the petroleum ether-insoluble extract from *Annona reticulata* seed (Y 10) against the eggs of *Plutella maculipennis*, the highest concentrations (1·6 and 0·8% w/v) showed some effect. A fairly high percentage of larvae developed normally until the larva died, leaving the egg shell as in hatched eggs but with the dead and shrivelled larva inside. A similar test against the eggs of *Ephestia kühniella* (Mediterranean flour moth) failed owing to the high death-rate in the controls.

Conclusions

It has been shown that by extraction with ether and precipitation from petroleum ether the insecticidally potent principle of *Annona reticulata* and *squamosa* seeds and roots can be concentrated from fifty- to one hundred-fold. This resinous concentrate has the properties of a glyceride of a hydroxylated unsaturated acid or acids of high molecular weight. By saponification the free acids have been obtained which likewise are highly toxic.

These extracts have been examined for contact and stomach poison and ovicidal properties in a variety of media. As a contact insecticide against *Aphis fabae*, *Macrosiphoniella sanborni* and *Macrosiphum solanifolii* the toxicity of the concentrate was of the same order as that of rotenone although of a delayed type; but was

considerably less toxic to *Oryzaephilus surinamensis*. The potency is therefore of a more restricted nature than that of rotenone although highly effective against aphides. As a stomach poison the extract was both toxic and repellent to *Plutella maculipennis* larvae; but was neither toxic nor repellent to *Diataraxia oleracea* larvae. Ovicidal tests against the eggs of *Plutella maculipennis* and of *Ephestia kühniella*, although showing some effect, were inconclusive.

Our thanks are due to Dr F. Tattersfield for his interest, help and encouragement, to Mr M. J. Way for carrying out stomach poison trials for us and to the Ministry of Agriculture and the Colonial Development Fund for grants towards the cost of the investigation, to the Director of Agriculture in Ceylon for a supply of *Annona* species.

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THIOCYANATE INSECTICIDES: THE RELATION BETWEEN KNOCK-DOWN ACTIVITY AND CHEMICAL CONSTITUTION

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The results of an investigation into the relation between chemical constitution and the knock-down activity and toxicity of thiocyanate insecticides are recorded. Linkage of the toxic thiocyano radical through a ketomethylene group to a lipoid soluble hydrocarbon residue gives rise to knock-down activity. The most active α-thiocyanoketones $R.CO.CH_2.SCN$ and thiocyanoacetates $R.O.CO.CH_2.SCN$ are too irritant to the eyes and nose for inclusion in domestic fly-sprays. Thiocyanoacetates derived from saturated 10-carbon alcohols, however, are non-irritant to the mucous membranes while possessing moderately high activity.

The mode of action of these compounds is discussed.

Introduction

Rapid knock-down action is an essential requirement of commercial fly-sprays. Some of the more recently developed insecticides, particularly D.D.T. and 'Gammexane', are exceptionally toxic but possess poor knock-down properties, and when these compounds are made the toxic basis of fly-sprays, the required degree of knock-down activity is normally conferred by the addition of pyrethrins.

In the course of a search for synthetic substitutes for the pyrethrins, in short supply during the war, a range of thiocyanoacetates $R.O.CO.CH_2.SCN$ was prepared and these compounds were evaluated against the house-fly. Thiocyanoacetates are, in general, free from the unpleasant smell characteristic of the alkyl thiocyanates and, in addition to their rapid knock-down action, are also highly toxic to house-flies. The most active compounds were too irritant to the mucous membranes to have any practical value as constituents of fly-sprays, but those obtained from a saturated aliphatic or alicyclic alcohol containing 10 carbon atoms were sufficiently active, and at the same time non-irritant. In the course of this work, a commercial preparation marketed by the Hercules Powder Co. under the name 'Thanite' was also examined. The active principle is stated to be the thiocyanoacetate of a secondary terpene alcohol.

The knock-down activity of the alkyl thiocyanates is low. In thiocyanoacetates, therefore, the —SCN radical is not the only cause of activity, and if the function of the radical *R*— is to give to the molecule as a whole the necessary solubility in

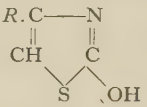
lipoids, then the ester ketomethylene linkage between the hydrocarbon and —SCN radicals must be responsible in part for the activity. Accordingly, a series of α-thiocyanoketones of general formula $R.CO.CH_2.SCN$ were prepared, but while these substances were more active than the corresponding thiocyanoacetates, they were also more irritant. This seemed to indicate that activity might be connected with the degree of enolization of the ketomethylene group

$$R\text{—}CO\text{—}CH_2.SCN \rightleftharpoons R\text{—}\overset{\overset{OH}{|}}{C}\text{=}CH.SCN,$$

and the possibility arose that the grouping

$$R_1\text{—}\overset{\overset{R_2}{|}}{C}\text{=}CH.SCN,$$

in which the —SCN radical is attached directly to an ethylenic double bond might be essential to knock-down action. A number of compounds of general formula $R_1R_2C{=}CR_3.SCN$ were synthesized and evaluated, but all proved to be completely inactive. α-Thiocyanoketones when treated with alkalis or mineral acids readily undergo rearrangement to give the isomeric 4-substituted-2-hydroxythiazoles



which bear some structural resemblance to the thiazole portion of vitamin B_1; and it was thought that the activity of the ketones might arise from interference with some internal process involving this vitamin. However, two hydroxythiazoles prepared from the most active α-thiocyanoketones were found to be without knock-down activity when applied externally in the form of a fly-spray.

EXPERIMENTAL

Thiocyanoacetates (Table 1) were prepared from suitable alcohols and phenols in two stages:

(1) Esterification of the alcohol or phenol to give the chloroacetate.

(2) Reaction of the chloroacetate with potassium thiocyanate.

(1) *Preparation of alcohols*

(*a*) *The reaction of ethylene oxide with certain higher alcohols.* The alcohols were heated with equivalent quantities of metallic sodium and ethylene oxide in sealed tubes at 80° C., the products of reaction shaken with water and the oils separating dried and fractionated under reduced pressure. By this procedure 2-caproxy-ethanol, b.p. 96°/13 mm., 2-fenchoxy-ethanol, b.p. 100°/6 mm., and 2-(3-(α-tetra-hydrofuryl)-1 methyl-*n*-propoxy)-ethanol, b.p. 100°/4 mm., were obtained.

(*b*) 4-(ααβγ-tetramethyl-*n*-butyl)-cyclohexanol, b.p. 117–27°/2 mm., was prepared from crude 4-(ααβγ-tetramethyl-*n*-butyl)-phenol by hydrogenation at 160° C.

and 50 atm. pressure in the presence of a Raney nickel catalyst. Under the same experimental conditions 4-cyclohexyl-butanol-(2), b.p. 120–4°/16 mm. (Vavon, 1912), and a mixture of *cis*- and *trans*-4-*tert*.-butyl-cyclohexanols (Darzens & Rost, 1911) were obtained in good yield from benzal acetone and 4-*tert*.-butyl-phenol respectively.

(2) *Preparation of the chloroacetates*

(*a*) *The action of chloroacetylchloride on phenols.* The phenol was refluxed 3 hr. with a 30% excess of chloroacetyl chloride. The excess of chloroacetyl chloride was then distilled off and the residual oil fractionated under reduced pressure.

(*b*) *Direct interaction of alcohols and chloroacetic acid.* Equivalent weights of the alcohol and chloroacetic acid were heated together from 8 to 14 hr. at 160–80° C., the water produced being allowed to distil off through a wide air condenser. The best yields were obtained at the higher temperature with the longer time of heating and averaged 80% theoretical. The crude chloroacetate obtained after washing with water was purified by distillation *in vacuo*.

(3) *Preparation of the thiocyanoacetates*

The chloroacetates were refluxed 6 hr. with a 100% excess of potassium thiocyanate in ethanol. The products were washed with water, extracted with ether, dried, and the ether removed. In general, no further purification was attempted and, except where stated in Table 1, samples of the crude materials were submitted for the biological tests. The crude thiocyanoacetates were usually yellow or reddish brown oils and were, in fact, of a fair degree of purity as shown by the analytical results in Table 2. 4-*tert*-Butyl-cyclohexyl-thiocyanoacetate was tested both in the crude (sample code no. 12) and in the pure state (no. 11).

α-*Thiocyanoketones* (Table 3)

(1) *Preparation of the ketones.* Tetrahydroionone. Hydrogenation of β-ionone at 100° C. and 50 atm. pressure in the presence of a Raney nickel catalyst gave 1:1:3-trimethyl-2-(γ-hydroxy-butyl)-cyclohexane, b.p. 137–9°/2 mm., in good yield. The secondary alcohol was then oxidized using acidified potassium dichromate, the reaction temperature being kept below 65°, and the product washed with 5% NaOH, dried and distilled. Tetrahydroionone was obtained as a colourless oil, b.p. 133–7°/13 mm.; semicarbazone from ethanol, m.p. 178–9° C. (Ruzicka, 1919).

4-*tert*.-Butyl-cyclohexanone, colourless plates from ether, m.p. 50° C., semicarbazone, m.p. 214° (found: N 20·4%, $C_{11}H_{21}ON_3$ requires N 19·9%); and 4-cyclohexyl-butanone-(2), a colourless oil, b.p. 105°/12 mm., semicarbazone colourless plates from ethanol, m.p. 183° (found: N 19·2%, $C_{11}H_{21}ON_3$ requires N 19·9%), were prepared by oxidizing 4-*tert*.-butyl-cyclohexanol and 4-cyclohexyl-butanol-(2) according to the procedure outlined above. 4-*tert*. Butyl-cyclohexanone has hitherto only been described in the literature as a liquid, b.p. 106–8/18 mm. (Darzens & Rost, 1911).

TABLE 1. *Thiocyanoacetates: formulae and properties*

Code no.	Thiocyanoacetate $R.O.CO.CH_2.SCN.R$—	Formula	Boiling range of chloroacetate intermediate	Physical properties of thiocyanoacetate when purified
5	Capryl-	$C_{11}H_{19}O_2NS$	108–18/7 mm.	Colourless oil, b.p. 154–8/5 mm.
13	Lauryl-	$C_{15}H_{27}O_2NS$	140–60/1 mm.	Colourless oil, b.p. 188–90/1 mm.
3	Cyclohexyl-	$C_9H_{13}O_2NS$	98–101/12 mm.	—
8	Fenchyl-	$C_{13}H_{19}O_2NS$	129/10 mm.	—
6	Carvomenthyl-	$C_{13}H_{21}O_2NS$	120–7/4 mm.	—
9	Decahydro-2-naphthyl-	$C_{13}H_{19}O_2NS$	155–65/4 mm.	—
10	1-Methyl-3-cyclohexyl-*n*-propyl-	$C_{13}H_{21}O_2NS$	128/2 mm.	—
11, 12	4-*tert.*-Butyl-cyclohexyl-	$C_{13}H_{21}O_2NS$	148–50/13 mm.	Colourless needles, m.p. 48° C.
14	4-($\alpha\alpha\beta\gamma$-Tetramethyl-*n*-butyl)-cyclohexyl-	$C_{17}H_{29}O_2NS$	163–70/5 mm.	—
15	Phenyl-	$C_9H_7O_2NS$	120–30/13 mm.,* m.p. 44° C.	m.p. 29° C.*
16	α-Naphthyl-	$C_{13}H_9O_2NS$	165–8/3 mm.,† m.p. 50° C.	—
17	4-($\alpha\alpha\beta\gamma$-Tetramethyl-*n*-butyl)-phenyl-	$C_{17}H_{23}O_2NS$	169–79/8 mm.	—
18	Tetrahydrofurfuryl-	$C_8H_{11}O_3NS$	110–15/2 mm.	—
19	1-Methyl-3-(α-tetrahydrofuryl)-*n*-propyl-	$C_{11}H_{17}O_3NS$	130–40/8 mm.	—
20	$\beta\beta'$-Di-(α-tetrahydrofuryl)-diethylcarbinyl-	$C_{16}H_{25}O_4NS$	175–85/1 mm.	—
23	2-Caproxyethyl-	$C_{13}H_{23}O_3NS$	105–10/6 mm.	—
24	2-Fenchoxyethyl-	$C_{15}H_{23}O_3NS$	130–5/10 mm.	—
25	2-(1-Methyl-3-(α-tetrahydrofuryl)-*n*-propoxy)-ethyl	$C_{15}H_{25}O_3NS$	135–45/7 mm.	—
26	2-β-Naphthoxyethyl-	$C_{15}H_{13}O_2NS$	m.p. 68–72° C.	Colourless needles, m.p. 95° C.

* Wheeler & Johnson (1901) give b.p. 123–8/13 mm. for phenyl chloroacetate and m.p. 28–32° for phenyl thiocyanoacetate.

† Ullmann (1897) gives m.p. 48° C.

Samples of methyl-, *n*-hexyl-, 2-ethyl-hexyl-, 2-methoxy-ethyl-, 2-butoxy-ethyl-, and 2-(2-ethoxy-ethoxy)-ethyl thiocyanoacetates were kindly supplied by Dr W. W. Smith.

TABLE 2. *Composition of the thiocyanoacetates*

Code no.	Thiocyanoacetate $R.O.CO.CH_2.SCN.R$—	Found %				Required %			
		C	H	N	S	C	H	N	S
3	Cyclohexyl-	54·5	6·8	7·0	15·9	54·2	6·6	7·0	16·1
9	Decahydro-2-naphthyl-	62·0	7·7	6·0	12·4	61·6	7·6	5·5	12·7
23	2-Caproxyethyl-	57·5	8·5	5·8	14·5	57·1	8·5	5·1	11·7
12	4-*tert.*-Butyl-cyclohexyl-	62·5	8·8	6·0	14·4	61·1	8·3	5·5	12·6
11	Purified 4-*tert.*-butyl-cyclohexyl-	61·2	7·9	5·5	12·5	61·1	8·3	5·5	12·6

ω-Chloro-4-methoxy-acetophenone, m.p. 102°, was obtained from anisole and chloroacetyl chloride by the Friedel-Crafts reaction (Kunckell & Johannsen, 1897).

(2) *Halogenation.* The ketones were halogenated (usually brominated) at 25–30° C. in the presence of calcium carbonate and water according to the method of Kotz & Steinhorst (1911), and the crude oils obtained washed with 5% sodium carbonate solution and distilled under reduced pressure.

(3) *Thiocyanation.* The α-halogeno-ketones were refluxed 6 hr. with a 100% excess of potassium thiocyanate in ethanol, and the crude thiocyanoketones obtained were usually tested without further purification. Crude ω-thiocyanoacetophenone and ω-thiocyano-4-methoxy-acetophenone were recrystallized from ethanol, and the melting-points of the purified compounds found to be in good agreement with those reported by Rabcewicz-Zubkowski & Kaflinska (1930).

TABLE 3. *Thiocyanoketones: formulae and properties*

Code no.	Compound	Formula	Boiling range of α-bromo intermediate	Physical properties of thiocyanoketone when purified
28	Thiocyanoacetone	C_4H_5ONS	40/13 mm.	Colourless oil, b.p. 112°/7 mm.
33	2-Thiocyanocyclohexanone	C_7H_9ONS	90–110/14 mm.	—
34	2-Thiocyano-4-*tert.*-butyl-cyclohexanone	$C_{11}H_{17}ONS$	110–20/3 mm.	—
29	Thiocyano-4-cyclohexyl-butanone-(2)	$C_{11}H_{17}ONS$	110–40/3 mm.	—
30	Thiocyanotetrahydroionone	$C_{14}H_{23}ONS$	Not distilled	—
31	ω-Thiocyanoacetophenone	C_9H_7ONS	Phenacyl-chloride, m.p. 54–5° C.	Colourless needles, m.p. 73°
32	ω-Thiocyano-4-methoxy-acetophenone	$C_{10}H_9O_2NS$	4-Methoxy-phenacyl-chloride, m.p. 102° C.	Colourless needles, m.p. 123–4°

Miscellaneous. 5-Thiocyano-2:3-dihydropyran and ethyl thiocyanofumarate were obtained as a result of refluxing the corresponding chloro-compounds with an excess of potassium thiocyanate in ethanol. Heating 1-chloroheptene-(1) with potassium thiocyanate in the presence of a copper catalyst gave crude 1-thiocyano-heptene-(1) in poor yield. 4-Phenyl-2-hydroxythiazole, long yellowish needles, m.p. 208° C., was prepared from ω-thiocyanoacetophenone by the action of concentrated hydrochloric acid according to the method of Hantzch (1927), and 4-methyl-2-hydroxythiazole, colourless needles, m.p. 104°, from thiocyanoacetone according to Tcherniac (1892).

Biological methods

An examination of the knock-down activity of the compounds under review was made on the house-fly, *Musca domestica* L., in a small spray chamber ($2 \times 2 \times 4\frac{1}{2}$ ft.) constructed of plywood and finished internally with a hard, glossy enamel. At one end a small electric fan was fitted, and at the opposite end was a detachable door. In the roof and in one side of the chamber were small glass observation windows. After the liberation into the chamber of 80–100 house-flies, spraying was carried out with an Aerograph spray gun, type MP, which was operated through a hole in

the end of the chamber behind the fan, 4 c.c. of spray being used in each test. Between every two tests the walls of the chamber were wiped over with a mixture of alcohol and acetone, and clean white paper was laid over the floor. Observations on the knock-down of flies were made 2·5, 5, 7·5 and 10 min. after the spraying was finished and the fan set in motion.

Irritancy tests were carried out by spraying a small quantity of liquid into the air in front of the face and recording the action of the mist on the mucous membranes of the eyes and nose.

A few of the compounds were examined for toxicity in a Peet-Grady chamber by a method which was slightly modified from the standard Peet-Grady test (Peet & Grady, 1928).

The house-flies used in the tests were 2–3 days old. They were bred at 25–27° C. in glass jars on the following food mixture: 12 lb. bran, 6 lb. grass meal, 1 oz. dried blood, 1 oz. malt extract, 1¼ oz. dried yeast.

Results of biological tests

Knock-down tests

All compounds were tested at a concentration of 1·0% w/w in deodorized kerosene or deodorized kerosene + sufficient acetone to give complete solution. Three replicate experiments were made with each sample, and the means are recorded in Table 4. A code number is assigned to each compound to facilitate cross-reference.

Thiocyanoacetates $R.O.CO.CH_2.SCN$

The effect of increasing molecular weight on the knock-down activity and irritant action is apparent from an inspection of Tables 4 and 5.

Saturated compounds, whether aliphatic or cyclic, are more active than the corresponding aromatic compounds with the same number of carbon atoms. In the aliphatic and alicyclic series, the irritant effect on the mucous membranes decreases regularly with increasing molecular weight; R— must contain not less than 10 carbon atoms before a 1·0% solution of the thiocyanoacetate becomes non-irritant to the human eyes and nose. *n*-Hexyl-thiocyanoacetate is the most active of the aliphatic compounds examined, giving a 99% knock-down of flies in 7·5 min., while the phenyl and cyclohexyl compounds are outstanding in the aromatic and alicyclic series respectively. Further increase in the molecular weight of the radical *R*— brings about a diminution in activity. All the aromatic thiocyanoacetates tested are irritant; derivatives of alcohols containing the tetrahydrofuryl grouping, while sometimes of considerable activity (e.g. no. 25, 2-(1-methyl-3-(α-tetrahydrofuryl)-*n*-propoxy)-ethyl-thiocyanoacetate), have marked irritant effects. In the series of general formula $R'.O.CH_2.CH_2.O.CO.CH_2SCN$, where R' is a saturated hydrocarbon radical, maximum activity is reached with the compound having 6 carbon atoms in the radical R', and 1·0% solutions of these thiocyanoacetates become non-irritant when R' contains 10 carbon atoms.

TABLE 4. *Thiocyano-compounds: knock-down activities*

Code no.	Compound	% Knock-down in min. 2·5	5	7·5	10	Irritation
1	Methyl thiocyanoacetate	2	15	37	58	Irritant
2	*n*-Hexyl-	9	56	99	100	,,
3	Cyclo-hexyl-	26	47	76	99	,,
4	2-Ethyl-hexyl-	14	42	56	84	Slightly irritant
5	Capryl-	0	9	23	58	,,
6	Carvomenthyl-	3	48	64	91	Non-irritant
7	'Thanite' (96 % *iso*-borneyl-thiocyanoacetate)	14	42	65	93	,,
8	Fenchyl-	7	35	59	95	,,
9	Decahydro-2-naphthyl-	7	19	32	51	,,
10	1-Methyl-3-cyclohexyl-*n*-propyl-	2	13	28	60	,,
11	Purified 4-*tert.*-butyl-cyclohexyl-	1	10	23	78	,,
12	4-*tert.*-Butyl-cyclohexyl-	5	18	27	51	,,
13	Lauryl-	0	0	0	2	,,
14	4-(ααβγ-Tetramethyl-*n*-butyl)-cyclohexyl-	6	15	15	16	,,
15	Phenyl-	1	6	24	36	Irritant
16	α-Naphthyl-	0	5	7	14	,,
17	4-(ααβγ-Tetramethyl-*n*-butyl)-phenyl-	0	0	0	0	,,
18	Tetrahydrofurfuryl-	0	17	23	25	Very irritant
19	1-Methyl-3-(α-tetrahydrofuryl)-*n*-propyl-	2	42	61	91	,,
20	ββ′-Di-(α-tetrahydrofuryl)-diethylcarbinyl-	0	1	6	28	,,
21	2-Methoxyethyl-	14	16	27	35	Irritant
22	2-Butoxyethyl-	4	32	53	86	,,
23	2-Caproxyethyl-	5	35	49	88	Slightly irritant
24	2-Fenchoxyethyl-	23	46	73	90	Non-irritant
25	2-(1-Methyl-3-(α-tetrahydrofuryl)-*n*-propoxy)-ethyl thiocyanoacetate	40	61	78	93	Very irritant
26	2-β-Naphthoxyethyl-	0	0	0	5	Irritant
27	2-(2-Ethoxy-ethoxy)ethyl-	6	12	12	14	Slightly irritant
28	Thiocyanoacetone	25	41	69	91	Very irritant
29	Thiocyano-4-cyclohexyl-butanone-(2)	1	23	61	90	Irritant
30	Thiocyanotetrahydroionone	0	0	0	4	Non-irritant
31	ω-Thiocyanoacetophenone	49	74	82	98	Very irritant
32	ω-Thiocyano-4-methoxy-acetophenone	0	2	2	3	,,
33	2-Thiocyanocyclohexanone	21	40	47	57	Irritant
34	2-Thiocyano-4-*tert.*-butyl-cyclohexanone	0	0	0	6	,,
35	1-Thiocyanoheptene-(1)	2	4	4	7	Non-irritant
36	5-Thiocyano-2:3-dihydropyran	1	3	3	3	,,
37	Ethyl-thiocyanofumarate	1	1	1	2	,,
38	4-Methyl-2-hydroxy-thiazole	1	1	1	2	,,
39	4-Phenyl-2-hydroxy-thiazole	1	2	2	3	,,
	Acetone	0	1	1	1	
	Purified kerosene	0	0	0	0	

TABLE 5. *Knock-down activity of aliphatic and alicyclic thiocyanoacetates. Concentration* 1·0% *w/w*

Compound code no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
C atoms in radical *R*—	1	6	6	8	8	10	10	10	10	10	10	10	12	14
% Knock-down in 7·5 min.	37	99	76	56	23	64	65	59	32	28	23	27	0	15
Irritation	I	I	I	SI	SI	NI	NI	NI	NI	NI	NI	NI	NI	NI

I = irritant; SI = slightly irritant; NI = non-irritant.

α-Thiocyanoketones

Where direct comparison is possible, the thiocyanoketones tend to be more active than the corresponding esters (compare thiocyanoacetone (no. 28) with methyl thiocyanoacetate (no. 1); ω-thiocyanoacetophenone (no. 31) with phenyl thiocyanoacetate (no. 15)). Unfortunately, they are also more irritant. The variation of knock-down activity and irritant action with increasing molecular weight in the series of aliphatic and alicyclic ketones $R.CO.CH_2.SCN$ and $-R'.CO.CH(SCN)-$ is brought out in Table 6.

TABLE 6. *Knock-down activity of aliphatic and alicyclic thiocyanoketones*

Code no.	28	29	30	33	34
C atoms in *R*— or —*R'*—	1	8	11	4	8
% knock-down in 7·5 min.	69	61	0	47	0
Irritation	VI	I	NI	I	I

VI = very irritant; I = irritant; NI = non-irritant.

The exact number of carbon atoms in the radicals *R* and *R'* corresponding to maximum activity has not been ascertained with anything approaching accuracy but lies somewhere between 1 and 8. Compounds in this series do not become non-irritant at a concentration of 1·0% until *R* contains 11 carbon atoms. The marked decrease in irritation on passing from thiocyano-4-cyclohexyl-butanone-(2) (no. 29) to thiocyanotetrahydroionone (no. 30) is accompanied by an equally striking fall in knock-down activity. There is therefore little possibility of obtaining an active non-irritant α-thiocyanoketone: ω-thiocyanoacetophenone (no. 31) shows exceptional activity but is very irritant. The introduction of a methoxyl-group at the *p*-position of the aromatic nucleus has the rather surprising effect of completely eliminating the knock-down activity while leaving the irritability unaltered.

Miscellaneous

Substances (nos. 35–37) of general formula $R_1R_2C{=}CR_3.SCN$ in which the —SCN radical is attached directly to an ethylenic double bond show very little promise as knock-down agents, so also do both the substituted hydroxythiazoles (nos. 38–9) prepared incidentally from the most active of the thiocyano ketones (compare ω-thiocyanoacetophenone (no. 31) and 4-phenyl-2-hydroxy-thiazole (no. 39)). Rearrangement of the thiocyanoketone molecule has a profound effect, not only on the knock-down activity of the compound but also on its irritant action on the mucous membranes, both hydroxythiazoles being non-irritant.

Extension to higher concentrations

A number of thiocyanoacetates in Table 4 were selected on a basis of high knock-down power, non-irritability to the human eyes and nose, and solubility in kerosene, and retested at higher concentrations. The results obtained on these compounds, all of which were derived from saturated 10-carbon alcohols, are shown in Table 7.

Table 7. *Thiocyanoacetates: variation of knock-down activity and irritation with concentration*

Code no.	Thiocyanoacetate *R*—	Concentration (% w/w)	% knock-down in min. 2·5	5	7·5	10	Irritation
6	Carvomenthyl-	1	12	30	58	79	Slightly irritant
		2	34	50	76	100	Slight to moderate
		4	46	64	78	97	Irritant
9	Decahydro-2-naphthyl-	1	7	19	32	51	Non-irritant
		2	29	46	60	95	Slight irritation to eyes
		4	29	59	80	98	Irritant
12	4-*tert.*-Butyl-cyclohexyl-	1	5	18	27	51	Non-irritant
		2	15	31	55	93	Irritant
		4	13	52	63	97	„
11	Purified 4-*tert.*-butyl-cyclohexyl-	1	0	9	37	65	Non-irritant
		2	16	48	62	92	Slightly irritant
7	'Thanite'	1	8	34	52	75	Non-irritant
		2	23	56	69	96	Slightly irritant
		4	37	64	77	98	Irritant

Consideration of these results and those in Table 4 indicates little difference in the behaviour of the crude 4-*tert.*-butyl-cyclohexyl-thiocyanoacetate (no. 12) compared with the rigorously purified sample (no. 11). In all cases an increase in the concentration of the active material is accompanied by a corresponding increase in irritation; 'Thanite', non-irritant at a concentration of 1·0%, is slightly irritant at 2·0% and irritant at 4·0%. It is believed, nevertheless, that all the compounds in Table 7 could be used at a concentration of 2·0% without undue discomfort. At this concentration there is little to choose between any of the compounds tested; at 1·0%, thiocyanoacetates derived from terpene alcohols, e.g. carvomenthyl thiocyanoacetate, are decidedly superior. Decahydro-2-naphthyl-thiocyanoacetate and 4-*tert.*-butyl-cyclohexyl-thiocyanoacetate, however, are more readily accessible.

Peet-Grady tests

The results of some typical toxicity experiments are given in Table 8.

TABLE 8. *Toxicity of 'Thanite' and fenchyl thiocyanoacetate*

Code no.	Compound	Concentration (w/w)	% kill
7	'Thanite'	1·75	36
8	Fenchyl thiocyanoacetate	1·75	38
		2·5	62
		3·25	94
	Standard pyrethrum insecticide	0·1 % w/v total pyrethrums	38

Our results with 'Thanite' are substantially in agreement with those claimed by the manufacturers for this preparation.

DISCUSSION

The main conclusion to be drawn on the knock-down effect of thiocyanoacetates $R.O.CO.CH_2.SCN$ and α-thiocyanoketones $R.CO.CH_2.SCN$ is that activity in any given series is largely dependent on the number of carbon atoms in the radical *R*. This is brought out in Table 7 where the thiocyanoacetates of a variety of saturated 10-carbon alcohols are tested at a concentration of 2·0%. Nevertheless, activity is not entirely dependent upon molecular weight and there are certain well-defined differences between the knock-down activities of isomers (compare, for example, 2-ethyl-hexyl-thiocyanoacetate (no. 4) and capryl thiocyanoacetate (no. 5)). There seems to be little doubt that narcosis or knock-down involves indirect blocking of enzyme activity by absorption of the insecticide on the active surfaces (Warburg, 1927; Hurst, 1945). Knock-down action is thus visualized as an essentially physical process involving the molecule as a whole; there is, after all, little time for any complicated series of chemical reactions to occur. Before this mechanism can come into operation, the insecticide, to reach the site of action, has to penetrate the lipoprotein mosaic that goes to make up insect cuticle (Hurst, 1943*a*, *b*) and a certain solubility in lipoids is necessary. In the thiocyanoacetates and thiocyanoketones under review this lipoid solubility is provided by the hydrocarbon radical *R*—. In penetration problems of this type involving a distribution between heterogeneous phases a point of maximum activity will be reached on ascending a given homologous series provided that the rate of decrease of water solubility exceeds the rate of decrease of the distribution coefficient between the aqueous and non-aqueous phases (Ferguson, 1939). Beyond this point further members of the series are inactive. The position of the cut-off in a homologous series is therefore determined by the solubility characteristics of the insecticide molecule (especially the lipophobic portion) and by the particular insect under investigation. For the series of aliphatic and alicyclic thiocyanoacetates maximum activity is reached when *R*— contains approximately 6 carbon atoms, but the decrease in activity beyond this point is by no means as rapid as is sometimes found (see, for example, the antibacterial activity of 4-alkyl resorcinols against *B. typhosus* (Dohme *et al.* 1926)), and compounds containing a 10-carbon radical show considerable activity. Lauryl thiocyanoacetate (no. 13), however, is completely inactive; in striking contrast to the series of alkyl thiocyanates wherein the lauryl compound is the most toxic against a variety of insects (Bousquet *et al.* 1935). The position of maximum activity has not been determined for the aliphatic and alicyclic α-thiocyanoketones but there is a very sharp cut-off on passing from an 8-carbon to an 11-carbon radical. It is true that in the few cases where direct comparison is possible, α-thiocyanoketones $R.CO.CH_2.SCN$ are more active than the corresponding thiocyanoacetates $R.O.CO.CH_2.SCN$ containing the same number of carbon atoms in the radical

R—, and that this might indicate that knock-down activity was in some way connected with the degree of enolization of the keto-methylene group

$$R.CO.CH_2.SCN \rightleftharpoons R—\overset{OH}{\overset{|}{C}}=CH.SCN;$$

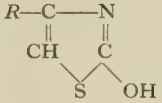
however, activity is of the same order in both series and on consideration of the sharp cut-off found between *R*=8 and *R*=11 on ascending the series of ketones, it seems doubtful whether the rule would hold for a 10-carbon radical. The apparent enhanced activity of the ketones compared with the esters may simply be due to a shift of the site of maximum activity towards a compound of lower molecular weight in the former series.

Although the majority of the compounds were tested in the crude state, there is little doubt that the irritant action on the mucous membranes is due to the chemicals themselves and not to any impurities in the samples. The specimens of capryl-, phenyl-, and 4-*tert.*-butyl-cyclohexyl-thiocyanoacetates were all rigorously purified without any noticeable change in the degree of irritation. Irritation in a homologous series of thiocyanoacetates decreases steadily with increasing molecular weight and this decrease is probably connected with the steady decrease in the water solubility of the molecule on ascending the series. A working generalization is that the hydrocarbon radical of a thiocyanoacetate should contain 10 or more atoms if it is to be non-irritant at a concentration of 1·0%.

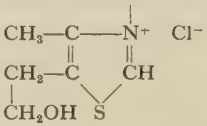
The mode of action of the thiocyanoacetates is of particular interest as this class of compound gives a good kill in addition to the characteristic narcotic (knock-down) effect. The histological effects of fly-sprays on the nerves and muscles of house-flies have been investigated by numerous workers, particularly Richards (1941–5), Hartzell *et al.* (1932–45), and Wilcoxon & Hartzell (1935). Insecticides having a knock-down action such as piperine (Hartzell & Strong, 1945) and the pyrethrins (for example Hartzell, 1934) give rise to characteristic lesions of the nerves of the central nervous system; no visible histopathological changes are apparent with rotenone and D.D.T. which are devoid of knock-down activity. The histopathological picture presented by 'Thanite' is similar to that seen with the pyrethrins though rather less marked, flies exposed for 10 min. to a 3·0% Thanite-kerosene spray suffering vacuolation of the larger nerve cells (Hartzell, 1945). Although Richards & Cutkomp (1945*a*) have shown the visible changes induced in nerves by insecticides to be largely post-mortem and therefore difficult to interpret, the effects must be due directly or indirectly to the action of the insecticide.

Alkyl thiocyanates and substances containing the —CH=CH.SCN grouping are inactive as knock-down agents so that the knock-down action of thiocyano-acetates and thiocyanoketones must be due to the absorption of these compounds on some enzyme system having particular affinity for the —CO.CH_2.SCN grouping.

While thiocyanoketones $R.CO.CH_2.SCN$ are readily converted into the isomeric substituted hydroxythiazoles



which are structurally related to the thiazole portion



of vitamin B_1, the inactivity of the two hydroxythiazoles tested would seem to throw some doubt on the possibility that knock-down activity might arise from the inhibition of some enzyme process involving this vitamin.

With regard to the toxic effects of thiocyanate insecticides two further possibilities are worthy of consideration. Von Oettingen *et al.* (1936) investigated the effects of alkyl thiocyanates on rats and mice both by subcutaneous injection and oral administration. With methyl and ethyl thiocyanates the effect on respiration simulated very closely that observed in cyanide poisoning. They found that pulped rabbit liver did in fact liberate HCN from the lower alkyl thiocyanates, 36·7% of the available HCN being produced from methyl thiocyanate in 48 hr. Any thiocyanoacetate might act as a source of methyl thiocyanate as a result of the following series of reactions:

$$R.O.CO.CH_2.SCN \xrightarrow{\text{hydrolysis}} ROH + HOOC.CH_2.SCN \text{ thiocyanoacetic acid}$$

$$HOOC.CH_2.SCN \xrightarrow{\text{decarboxylation}} CO_2 + CH_3SCN$$

The liberation of HCN inside the insect as the result of some further enzyme process would cause instant death by irreversible inhibition of the respiratory enzymes.

It has recently been shown (Ramos, 1944) that the effect of acetylcholine on the heart is accelerated and prolonged by thiocyanate. Thiocyanate acts by diminishing choline esterase activity and increasing the permeability of the cells to acetyl choline. Choline esterase is known to occur in insects (Richards & Cutkomp, 1945*b*), the interesting observation having been made that excess of substrate inhibits the enzyme (Augustinsson, 1946). The action of the alkaloid eserine (physostigmine) is known to be due to inhibition of choline esterase; thiocyanate may have a similar effect on the insect. Thus, while it is claimed that the relation between the chemical constitution and knock-down activity of thiocyanate insecticides has been clarified by the present investigation, the mode of action of these compounds is still obscure and requires further elucidation. Further work in this field is projected.

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PRIMULA OBCONICA, A CARRIER OF TOBACCO NECROSIS VIRUSES

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A tobacco necrosis virus has been isolated from the leaves and flowers of naturally infected *Primula obconica* plants. Although the virus produces no necrotic symptoms, it is not distributed uniformly through primulas, but occurs only in isolated regions, most of the tissues being apparently virus-free.

When inoculated to healthy primulas, three tobacco necrosis viruses were found to behave similarly. They all enter and multiply locally, but produce no symptoms; movement from the inoculated areas occurs only rarely and then does not cause a full systemic infection but only further localized infections. Multiplication of the viruses in primula is slower than in tobacco or French bean, which react necrotically.

The uncertainties in interpreting results of tests for tobacco necrosis viruses are described and possible explanations of natural infections are discussed.

Tobacco necrosis, which was named and shown to be a virus disease by Smith & Bald (1935), presents a number of novel features. It rarely occurs in the summer, but during the winter months it attacks large numbers of seedlings of *Nicotiana tabacum* and *N. glutinosa* raised under glass in conditions that prevent the spread of other virus diseases that have been studied in any detail. The symptoms of tobacco necrosis can be caused by several serologically unrelated viruses, which have different physical properties and stabilities (Bawden, 1941; Bawden & Pirie, 1942), but which share some unusual host-parasite relationships. Although these viruses have a wide host range, no hosts are known in which they produce a systemic infection, and symptoms in naturally infected plants have been noted only in tobacco and *N. glutinosa*. In these two species symptoms are restricted to leaves that are in contact with the soil, and the upper leaves not only look normal but also seem to be virus-free. The roots of such plants, however, are infected, and the viruses can also often be found in the roots of plants whose foliage has shown no symptoms. Indeed, this seems to be the usual condition in naturally infected plants of other species, for although the viruses have been found to occur in the roots of many different species, they have not been detected in the aerial parts (Smith, 1937*a*). This is not because the leaves are immune from the viruses. If the upper leaves of tobacco and *N. glutinosa*, or leaves of other susceptible species, are inoculated, they become infected. With two exceptions, all such species have reacted by the production of necrotic local lesions. The exceptions are *Primula obconica* and *Polyanthus* sp., which Smith (1937*a*) found to be 'resistant to infection when inoculated on the leaves,

although the virus is frequently present in the roots'. It is the purpose of this paper to record the occurrence of a tobacco necrosis virus in the leaves and flowers of naturally infected *Primula obconica* and to describe in more detail the behaviour of this plant when infected.

Material and methods

Infectivity tests for tobacco necrosis viruses were made by inoculation to tobacco, var. White Burley, and French bean (*Phaseolus vulgaris*, var. Canadian Wonder). Tobacco reacts by producing white necrotic rings or spots. In French bean infection is less strictly localized than in tobacco; infection first shows by the production of brown or black spots, from which the necrosis spreads throughout the network of veins, when the inoculated leaf usually abscinds. Occasionally there is some necrosis of the petiole or stem, and if a concentrated inoculum has been used, especially on young plants, a necrotic lesion or two may appear on an upper, uninoculated leaf. Both tobacco and French bean become increasingly resistant with age. Tobacco seedlings were therefore used when in the four- to five-leaf stage, and the first-formed leaves of beans were inoculated about 10 days after sowing, before the trifoliate leaves were developed. The leaves to be inoculated were first lightly dusted with some abrasive, usually kieselguhr (Kalmus & Kassanis, 1945), as this greatly increases the ease of transmission, and were then rubbed over their whole surfaces with the fore-finger dipped in the inoculum. Serological tests were made by methods previously described (Bawden, 1941).

The *Primula obconica* plants that formed the starting material for this work were sent to us by Dr W. M. Ware. They came from a nursery in Folkestone where considerable losses of ridge cucumbers had been experienced because of cucumber mosaic virus, of which Dr Ware suspected that the *P. obconica* might be the source. Seven plants were received, four of which looked normal, one was suspicious and the other two obviously diseased. The last two were stunted, their leaves showed a definite mosaic and the flowers were broken. When sap from these two was inoculated to tobacco, *Nicotiana glutinosa* and cucumber, a few local lesions were obtained and 8 days later all the plants developed systemic symptoms typical of those caused by the common form of cucumber mosaic virus. Sap from the suspicious-looking *Primula obconica* produced rather more local lesions, and systemic symptoms did not develop on the test plants for more than a fortnight, and then on some only. These systemic symptoms started as patchy chlorotic spots and developed later into a bright yellow mottle. By plant protection tests, this virus was subsequently also identified as a strain of cucumber mosaic virus. Two of the four normal-looking *P. obconica* produced no reaction on the test plants and, presumably, were uninfected. Inoculum from the other two seemingly healthy plants produced white necrotic local lesions on all three species of test plants, none of which, however, developed any systemic symptoms. Virus produced by further inoculations from these lesions provided the material for further work.

A TOBACCO NECROSIS VIRUS IN THE FOLIAGE OF NATURALLY INFECTED PRIMULAS

The local lesions produced by inoculum from the leaves of all five infected *P. obconica* were of the same type and were similar to those pictured by Ainsworth (1935) as caused by strains of cucumber mosaic virus he isolated from monocotyledonous plants. As three of the *P. obconica* plants were known to be infected with cucumber mosaic virus, it seemed reasonable to suppose that the range of reactions obtained on the test plants was explicable by individual *P. obconica* containing different strains of cucumber mosaic virus, which varied from one another in their ability to cause necrosis and to remain localized in the test plants. This idea, however, became untenable when it was found that tobacco plants systemically infected with cucumber mosaic virus were still susceptible to the virus causing the local lesions, for Price (1935) and Ainsworth (1938) have shown that strains of this virus that cause a systemic mottle protect plants against subsequent infection with strains that cause local necrosis. It then seemed possible that the local lesions were caused by a tobacco necrosis virus, for which *P. obconica* was a systemic host.

This interpretation became increasingly probable when the virus was inoculated to French bean, for it caused the characteristic dark-coloured and spreading necroses. The results of a thermal inactivation test also further suggested that a tobacco necrosis virus might be involved, and they excluded the possibility of a strain of cucumber mosaic virus. Sap from infected tobacco leaves was heated for 10 min. at various temperatures, and then inoculated to French bean. The unheated control produced an average of 175 lesions per leaf, whereas those heated at 60, 70 and 80° C. gave an average of 11, 3 and 1 lesions respectively. Thus the thermal inactivation point is above 80° C., far higher than that of cucumber mosaic virus, but there is much loss of infectivity when heating is carried out at temperatures well below the thermal inactivation point. This high thermal inactivation point, coupled with a small temperature coefficient for inactivation, has previously been shown to be a property of tobacco necrosis viruses (Price, 1940; Bawden, 1941).

Attempts were now made to identify the tobacco necrosis virus more closely. Single local lesions were taken from infected tobacco plants, ground up and inoculated separately to healthy plants. It was noticeable that fewer local lesions developed on the inoculated leaves than is usual when this technique is applied to the Rothamsted or potato cultures of tobacco necrosis viruses, and this suggested that the virus concerned might be one that multiplies less readily than these. Sap from the inoculated plants was clarified by freezing, thawing and centrifuging at 10,000 r.p.m., and then tested for its reaction with antiserum to the Rothamsted, potato and tobacco cultures of tobacco necrosis viruses (Bawden & Pirie, 1942). No precipitation occurred with any serum, a result that could be interpreted in two ways. It could mean that the virus was serologically unrelated to those previously identified or that it was present in too small a concentration to give a visible precipitate. If the latter were true, the virus from *P. obconica* was most likely to be related to the tobacco

culture, for sap from plants infected with this often fails to react with antiserum because of the low virus content. Further plants were inoculated to produce larger quantities of sap and the virus was concentrated ten times by precipitation with ammonium sulphate, and the precipitin tests repeated. This time a positive reaction was obtained with the antiserum to the tobacco culture of Bawden and Pirie, while the other two sera gave no precipitation. Thus the relationship of the virus from *P. obconica* with one previously found in a plant suffering from tobacco necrosis was established. Whether the two are identical or related strains, however, remains undecided. Bawden & Pirie (1942) found that the serologically related viruses in the cultures they called tobacco I and tobacco II readily crystallized in a variety of crystal forms. We have been unable to obtain any crystals from the serologically related virus from *P. obconica*. The virus has been purified several times by precipitation methods and ultra-centrifuging, but it has consistently failed to produce crystals in conditions in which tobacco I and tobacco II did. Strict comparisons of the viruses under identical conditions could not be made, as the cultures of tobacco I and tobacco II are no longer maintained in the glasshouses. In their other properties, such as serological titre, yield and greater solubility in ammonium sulphate solutions at 0° than at 20° C., they are indistinguishable.

The presence of a tobacco necrosis virus in the original *P. obconica* was confirmed on many subsequent occasions when inoculations were made to French bean or tobacco. However, the results of these were far from consistent and suggested that the virus was not uniformly distributed throughout the plants as is customary in systemic infections of long standing. Inoculations from the same *P. obconica* might at one time produce forty or more lesions per leaf on the test plants, whereas at other times they produced none or only one or two. A systematic examination of the distribution of the virus in one of the plants was therefore made, sap from individual leaves and a flower being inoculated separately to French bean. Within this test, there was the same erratic behaviour; sap from the petals and two leaves gave considerable numbers of lesions, sap from another two leaves gave two or three lesions and from the other nothing. The petals of the flower taken showed some white rings. Only a few of the flowers showed rings and, as the plant used was free from cucumber mosaic virus, it was thought that they might be a symptom caused by the tobacco necrosis virus. Later tests, however, failed to confirm this, for no virus could be detected in inoculum taken from other flowers (from the same and other plants) showing similar rings. The results of all such tests suggested that the virus was irregularly distributed in the plants, occurring only in isolated areas often remote from one another.

Transmission of tobacco necrosis viruses to primulas

To gain more information about the multiplication, movement and distribution of tobacco necrosis viruses in *P. obconica*, seedlings were raised and inoculated. In the first test two leaves on each of five seedlings were inoculated with the tobacco

necrosis virus isolated from the naturally infected primula. None of the plants developed any symptoms, either on the inoculated leaves or on the uninoculated upper leaves. The virus entered and multiplied in the inoculated leaves, for when a fortnight later portions of these were ground up and inoculated to French bean more than 200 lesions per leaf were produced. A month after the *P. obconica* were inoculated, the test was repeated, this time inoculum being taken from both the inoculated and uninoculated leaves. Again sap from all the inoculated leaves produced large numbers of lesions, but that from uninoculated leaves of only one of the five plants gave any reactions. It produced an average of forty lesions per bean leaf, indicating a much smaller virus content than that of the inoculated leaves. After a further month, all the young leaves from this one plant were picked and sap from each leaf was inoculated separately to French bean. Again the results did not suggest a true systemic infection, but rather a patchy distribution of virus, for sap from only one of the leaves produced any lesions. Whether this virus had spread from the inoculated leaves or had originated because of some independent infection was uncertain. If it were the result of a chance infection, then it might well be a different virus from that inoculated to the *P. obconica*. The virus obtained from the uninoculated leaf was therefore propagated in tobacco plants, concentrated and purified, and tested serologically. It reacted only with antiserum to the tobacco culture of tobacco necrosis viruses, and in all its other properties resembled the virus originally inoculated. Thus it seems probable that virus did move out of the inoculated leaves, though this movement did not lead to a systemic invasion of all the young tissues.

Similar results were obtained when *P. obconica* plants were inoculated with other tobacco necrosis viruses. There was local multiplication of the viruses in the inoculated leaves, which showed no symptoms, and three months after infection sap from the inoculated leaves still produced more than 200 lesions per French bean leaf. From fifteen *P. obconica* seedlings inoculated with the Rothamsted and potato cultures of tobacco necrosis viruses, however, virus was detected in the uninoculated leaves of only two plants. The virus obtained from these leaves was serologically related to that with which the lower leaves were previously infected. Although many tests were made on leaves of control, uninoculated *P. obconica* growing in the same glasshouses, no virus was obtained. The roots of the three plants in which virus was detected in uninoculated leaves were also infected, but this probably has little significance. No attempt was made to relate the viruses obtained from the roots with those inoculated to the leaves, and virus was also found in the roots of some control plants as well as in the roots of some of the inoculated plants in which there was apparently no spread of virus in the tops. This occurrence of virus in the roots and seeming absence from the tops is general in many species, and it seems to be the common condition in *P. obconica* also. This fact makes the occurrence of virus in detectable amounts in the leaves of five out of the seven plants originally obtained from Folkestone appear an anomaly for which no explanation can be offered.

From tests on the virus content of the roots, it seems that the distribution of the virus in them is of the same irregular type as in the aerial parts. When roots of individual *P. obconica* plants were divided into several portions, sap from some portions often gave large numbers of lesions on the test plants, whereas that from others gave few or none. Similar results have also been obtained with tobacco and bean plants. There seems also to be little movement of viruses from the entry points in roots. Beans were grown in water culture solutions and their roots were rubbed with infective sap at various points. There was local multiplication of the virus, which showed by the production of a brown discoloration at the site of infection. This suggested a local lesion and there seemed to be little movement of virus away from the entry points. Portions of roots taken from both above and below the inoculated areas and tested some weeks after inoculation produced no lesions. Hence it seems probable that the total virus content of infected roots is an indication of the number of separate infections, and that these are mostly local and independent. This view, rather than that virus enters at one point and then becomes systemic in the root system, is in keeping with the wide range of virus content found in different parts of the same root and with the fact that different tobacco necrosis viruses can often be found in the roots of one plant (Bawden & Pirie, 1942).

Infected *Primula vulgaris* and *P. malachoides* seem to behave in much the same way as does *P. obconica*. When seedlings of these species were inoculated with three serologically unrelated tobacco necrosis viruses, there was local multiplication of the viruses without production of symptoms, but no virus was recovered from the uninoculated leaves of the five plants that were tested.

The difficulty with which tobacco necrosis viruses move in primula leaves was further shown by experiments in which only parts of individual leaves were inoculated. In some leaves the basal halves only were rubbed, and in others the apical halves. Forty days later, the inoculated leaves were picked, and the basal and apical halves were ground up separately and tested on French beans. All the halves that had been inoculated produced 100 or more lesions per leaf, whereas none of the other halves, whether basal or apical, produced more than four lesions and some gave none. Thus it would seem that in *P. obconica* leaves which show no symptoms, there is less movement of the viruses from original entry points than there is in French bean leaves showing necroses, for in the latter the viruses will move along the veins and spread over the inoculated leaf. The inability of the viruses to move freely through *Primula* sp. cannot be attributed to some peculiarity of the host, but seems to be restricted to tobacco necrosis viruses. Cucumber mosaic virus, for example, is commonly found in *Primula* sp. in which it produces a normal systemic infection and can regularly be recovered from any tissues. Similarly, we have found that tomato bushy stunt and tobacco ringspot viruses, which more nearly resemble tobacco necrosis virus than does cucumber mosaic virus, can both cause a systemic infection of *P. obconica*, the first causing a severe systemic necrosis and the second only a transient mottle.

The tobacco necrosis viruses multiply more slowly in *P. obconica* than in other species we have studied. In tobacco and French beans lesions appear in from 2 to 4 days after infection and the virus content of sap is maximal in about a week. By contrast, when daily tests were made from inoculated leaves of *P. obconica*, there was little evidence of virus multiplication until about the ninth day. Until then the average number of lesions per leaf on the test plant was only 6, on the ninth day it was 25, on the twelfth it was 60 and on the fourteenth was over 100. This fact probably explains Smith's (1937*a*) conclusion that *Primula* sp. are resistant to tobacco necrosis viruses, for he tested his inoculated plants after only one week. Another contributing factor is that Smith used no abrasive either in his inoculations to primulas or in making his tests, and this would greatly reduce the number of lesions obtained. In some of our tests from primulas, sap was inoculated to tobacco and bean leaves with and without the addition of kieselguhr. Lesions were often obtained on leaves that were dusted with kieselguhr, when there was none on the controls, and inoculations made without the help of an abrasive usually failed to produce infection unless those with abrasive were producing about forty lesions per bean leaf.

Discussion

Local infections, without a subsequent systemic invasion of the host, are well known with many different viruses, but all those previously recognised have been associated with necrotic lesions. As viruses are believed to be obligate parasites, the phenomenon found a ready explanation, because the death of the host tissues around the entry points might be expected to prevent further multiplication and spread of the pathogen. However, the explanation was never entirely satisfactory as many viruses do become systemic in hosts in which they cause necrotic local lesions. Clearly, no such explanation can be offered for the localization of tobacco necrosis viruses in leaves of *Primula* sp., for these are symptomless carriers. For some unknown reason, unlike other viruses that have been studied, these are apparently unable to pass from cell to cell in inoculated leaves or to move freely into and through the vascular system of infected plants, even of those in which they cause no necrosis. Indeed, there is more movement of tobacco necrosis virus in bean leaves, which react necrotically, than in primula leaves, which do not. Occasionally there does seem to be movement of virus in both these plants from inoculated leaves to younger tissues, but in neither does this movement lead to a full systemic infection. Instead, it leads to the infection of isolated islets of tissue, the equivalent of local lesions though remote from the site of inoculation. In beans this spread can be detected because of the production of necrotic spots on uninoculated leaves, but in the symptomless primulas it is only obvious when transmission tests for the virus are made. Presumably infective doses of the viruses occasionally escape from the originally infected cells into the vascular system through which they are carried to the cells where they become fixed and multiply, but there is no constant flow of virus from infected areas as there is in most other virus infections.

Chance movement of virus from infected roots probably also accounts for the occurrence of virus in the tops of naturally infected *P. obconica*, though how the original infections originate is still unknown. The frequent presence of virus in the roots of plants whose leaves seem to be uninfected, and the restriction of symptoms in *Nicotiana* sp. to leaves in contact with the soil, both suggest that the viruses are soil-borne; it is likely that the viruses enter healthy plants from the soil when they come into contact with suitable abrasions. On this theory, the seasonal occurrence of tobacco necrosis in tobacco plants can be explained by the fact that the environment greatly influences the susceptibility of leaves to infection. When raised under conditions of low light intensity, inoculated plants produce many more lesions per leaf than when grown under full summer light. Also, in winter the seedlings grow more slowly and remain in a highly susceptible state for much longer than in summer, when they develop and mature rapidly. We have found no natural infections in the roots of plants growing in water culture solutions, and Smith (1937*b*) found none in plants grown on agar medium in sterile conditions, but they are frequent in plants raised in soil that has been subjected to partial sterilization that would inactivate the viruses. Hence, if the viruses are soil-borne, it seems that they are fairly readily reintroduced into the soil. This re-entry would be simply explained if, in addition to the susceptible higher plants, the viruses also have as hosts some members of the soil microflora, for the recolonization of the soil by such infected organisms would again establish the viruses. Our attempts to test this theory have given it no support. We have been unable to obtain the viruses from cultures of soil-inhabiting bacteria or to transmit them by such plant pathogens as *Thielaviopsis basicola* and *Rhizoctonia solani*.

The whole problem of the origin of infection with tobacco necrosis viruses, however, is greatly complicated by the uncertainty of results of tests for the presence of virus. A positive result clearly implies the presence of virus, but a negative result has no such certain meaning. The tissues under test may be virus-free and so be qualitatively different from those giving a positive test. On the other hand, they may be infected, but contain too little virus to give a positive result with the method of testing. Before tobacco necrosis viruses can be detected in roots, or in the leaves of such plants as *Primula obconica*, the virus content must exceed a minimum, which will vary with the method of testing. For instance, plants that give a negative result when tests are made by inoculations to oldish bean leaves may give a positive result if younger, more susceptible test plants are used. Similarly, plants that seem to be virus-free when transmission tests are made by ordinary rubbing methods often give a positive reaction when some suitable abrasive is incorporated in the inoculum. The use of such an abrasive increases the sensitivity of inoculation tests by almost 100 times, so that without an abrasive about 100 times as much virus is needed to produce a lesion as when abrasive is used. Even with our most sensitive methods of testing, something like 10^{-10} g. of virus per ml., that is, more than a million virus particles, is needed to give a lesion. It is, in fact, impossible to state categorically

that any plant is ever free from tobacco necrosis viruses; the most that can be said is that it does not give a positive reaction. The difference between such a plant and one that does give a positive reaction may only be a quantitative difference in their virus contents and not the qualitative difference that is usually assumed to exist between infected and virus-free plants. It is almost certain that many plants passed as virus-free in earlier work on tobacco necrosis were infected, for many of the tissues we have tested have given positive results only when an abrasive was used, and it is highly probable that even more sensitive methods would have detected virus where our tests failed. Until methods are developed that allow the detection of single virus particles, the value of negative results will be uncertain, and there remains the possibility that tobacco necrosis viruses are always present in certain plant tissues, though normally they occur in quantities too small to be detected by present methods. If this is so, then what we now assume to be the result of infection from external sources may instead be merely the production of these viruses in abnormally high quantities because of some local stimulus. Until some positive evidence is found for this theory, however, it seems reasonable to proceed along more orthodox lines and assume that plants containing tobacco necrosis viruses have become infected from without and differ qualitatively as well as quantitatively from seemingly uninfected plants of the same species. Nevertheless, it is important to realize that this, as yet, is only an assumption.

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THE EFFECT OF PLANT EXTRACTS ON THE GERMINATION OF THE CONIDIA OF *VENTURIA INAEQUALIS*

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Of 1915 flowering plants 23% gave extracts which completely inhibited the germination of the conidia of *Venturia inaequalis* under the experimental conditions used. The plants which gave active extracts are distributed throughout the Angiosperms, and there is no correlation with systematic position. The inhibitors were distributed generally in some plants and were localized in others, e.g. to the leaves.

Some extracts appeared merely to inhibit the germination of the conidia while others killed them.

Some inhibitors disappeared when the plants were dried, other dry material was still active when retested several months later.

Hedera Helix contains an inhibitor which is active when the extract is diluted 128 times.

Until recently only inorganic chemicals have been used as fungicides, but oil emulsions are now used successfully against gooseberry mildew, salicylanilide against tomato leaf mould, and organo-mercury compounds against cereal smuts and other diseases. The work of Montgomery & Moore (1938) and others indicates that some other organic compounds are highly fungicidal. It seemed possible that some organic constituents of plants might have fungicidal activity, and this survey was undertaken to see if plants could be found with activities sufficiently high to warrant further chemical investigation. The causal organism of apple scab, *Venturia inaequalis*, was used, and the laboratory method of Montgomery & Moore (1938), which had been developed for use with this fungus, was employed as a convenient and rapid means of sorting the promising extracts from the rest.

Experimental

The principle of the method was as follows:

A constant volume of plant extract was dried by exposure to the laboratory air on a constant area of clean glass slide, delimited by marking rings of equal diameter with a diamond pencil. The dried extract was rewetted with a constant volume of a conidial suspension of the fungus, containing a constant number of conidia per c.c. The conidia were suspended in glass-distilled water. The slides were left in moist chambers at room temperature and were examined microscopically for germination for three successive days. If the conidia had not germinated in that time, it was assumed that the extract contained either a contact inhibitor, or an inhibitor sufficiently soluble or sufficiently toxic to reach inhibitory concentration in the conidial suspension overnight. Control conidia in glass-distilled water never failed

to germinate overnight. The method was used as described by Montgomery & Moore with a few minor modifications as follows:

(1) *Cleaning the slides.* After washing with soap and water, the slides were freed of grease by boiling in concentrated sulphuric acid containing a little sodium nitrate. They were rinsed and boiled in distilled water, dried, and stored in absolute alcohol.

(2) *Washing the deposits.* Montgomery & Moore washed their fungicide deposits in order to discover those substances which would be too readily removed from the foliage by rain. In the present work the extract deposits were never washed before testing since it was only a preliminary survey.

(3) *Storage of deposits.* The conidial suspension was always applied within a few minutes after the extract had dried. Storage overnight at 20° C. was omitted as being inconvenient and unnecessary at this stage.

(4) *Assessing the results.* Owing to the large numbers of tests set up, it was impossible to assess the germination by counting. Instead, the extracts were classed into two groups, one corresponding with group I of Montgomery & Moore, in which there was either no germination at all, or at most a very few conidia with 'inhibited' germ tubes. The other group corresponded with groups IV and V, in which almost all the conidia had germinated. Extracts which gave intermediate results were included among the inactive extracts, as the results were often variable on repetition.

Plant extracts were prepared by grinding plant material with washed sand and sufficient glass-distilled water to make a stiff pulp, which was then squeezed through silk. Leaves, stems, flowers and roots whenever possible were ground together, although this entailed a risk that some inhibitors might be destroyed by interaction of the different parts. On the other hand, some inhibitors might have been activated in the mixture. The extracts were always used freshly prepared, and any sediment which may have settled was resuspended.

Some active extracts were tested to discover whether they were fungicidal or fungistatic. The extract was made in the usual way and was centrifuged. A sample was tested against fresh conidia to confirm its initial toxicity. A heavy conidial suspension was added to the remainder of the extract which was then divided between three tubes. After standing at room temperature overnight, one tube was centrifuged to throw down the conidia which were then washed in several changes of glass-distilled water. Finally the conidia were resuspended in 1 c.c. glass-distilled water and tests were set up as follows:

(*a*) 0·04 c.c. washed conidial suspension was dried on each ring on the slide and fresh conidia were added in redistilled water. These were readily distinguishable because conidia kept in plant extracts under these conditions were invariably darker than fresh conidia. Germination counts were made daily, and if the fresh conidia failed to germinate, inadequate washing of the overnight conidial suspension was deduced.

(*b*) 0·04 c.c. washed conidial suspension was dried on each ring and was rewetted with glass-distilled water.

(*c*) 0·04 c.c. washed conidial suspension per ring was placed in moist chambers immediately without drying.

The second and third tubes were tested in the same way, 1 and 2 days later, and generally gave similar results to those from the first tubes.

If the conidia in (*b*) and (*c*) germinated the extract was not completely fungicidal. If, however, there was good germination of the fresh conidia in (*a*) and no germination in (*b*) and (*c*) it seemed probable that the conidia had been killed by the extract.

Limitations of the method

Only one test fungus *Venturia inaequalis* was used, but many inhibitory extracts were seen to support the germination and growth of accidental contaminants such as *Penicillium* spp., *Aspergillus* spp., *Cladosporium* spp. and many other fungi. Probably only a few of the active plants listed will give extracts inhibitory to other plant-pathogenic fungi.

The results show only those extracts which inhibit conidial germination; mycelial growth was not necessarily inhibited and in at least one case (*Caltha palustris*), mycelium in the conidial suspension grew well while the conidia did not germinate.

Results

Of 1915 species tested, 23% gave inhibitory extracts. 178 families out of an approximate total of 305 were tested and 113 families contained active members. Forty-five of Engler's fifty-five Angiosperm orders were sampled, and thirty-three contained inhibitory members. The ten orders which were unsampled all contained only one family each, and the twelve orders with no inhibitory members were all either very small orders, or were inadequately sampled.

The most strikingly active groups were:

Centrospermae	particularly	Chenopodiaceae
Ranales	,,	Ranunculaceae
Rhoeadales	,,	Cruciferae
Rosales	,,	Saxifragaceae, Hamamelidaceae, Pittosporaceae
Umbellales	,,	Araliaceae, Cornaceae, Umbelliferae
Primulales	,,	Theophrastaceae, Myrsinaceae, Primulaceae
Ebenales	,,	Sapotaceae, Ebenaceae
Tubiflorae	,,	Solanaceae
Campanulales	,,	Compositae
Liliiflorae	,,	Liliaceae, Dioscoreaceae

Plants producing active extracts were therefore found throughout the groups of the flowering plants, and their occurrence was quite unrelated to their taxonomic position.

Active Plants

Most of the commoner British species in the list are referred to in the discussion.

ACERACEAE
Acer Pseudo-Platanus L.
AKANIACEAE
Akania rosaefolia
AMARANTHACEAE
Amaranthus Blitum L.
AMARYLLIDACEAE
Allium cepa L.
A. sativum L.
Alstroemeria aurantiaca D. Don
ANACARDIACEAE
Harpephyllum caffrum Bernh.
Pistacia palaestina Boiss.
Rhus lancea L.f.
Schinus terebinthifolius Raddi
Soriendeia madagascariensis DC.
ANNONACEAE
Annona muricata L.
Mischogyne michelioides Exell.
APOCYNACEAE
Allamanda cathartica L. var. *Hendersonii* Hort.
A. neriifolia Hook.
Dipladenia sanderi Hemsl.
Picralima Klaineana Pierre
Plumeria tricolor Ruiz & Pav.
ARALIACEAE
Hedera Helix L.
Meryta Sinclairii Seem.
Oreopanax guatemalense Decne. & Planch.
Pseudopanax crassifolium Seem. var. *trifoliatum* Kirk
ASCLEPIADACEAE
Vincetoxicum officinalis Moench
BALSAMINACEAE
Impatiens oliveri C. H. Wright
BEGONIACEAE
Begonia acutifolia Jacq.
B. Haageana Hort.
B. semperflorens Link & Otto
BETULACEAE
Alnus firma Sieb. & Zucc. var. *Sieboldiana* H. Winkl.
A. viridis Lam. & DC.
BIGNONIACEAE
Adenocalymna nitidum Mart.
BURSERACEAE
Bursera jorullensis Engl.
BUXACEAE
Sarcococca hookeriana Baill.
S. humulis Hort.
CAMPANULACEAE
Phyteuma canescens Waldst. & Kit.
CANELLACEAE
Canella Winteriana Gaertn.
CAPPARIDACEAE
Capparis jamaicensis Jacq.
Steriphoma ellipticum Spreng.
CAPRIFOLIACEAE
Lonicera Periclymenum L.
Symphoricarpos albus Blake
Viburnum macrocephalum Fortune
V. utile Hemsl.
CARYOPHYLLACEAE
Lychnis chalcedonica L.
L. diurna Sibth.
Silene Cucubalus Wibel
Spergula arvensis L.
CERCIDIPHYLLACEAE
Cercidiphyllum japonicum Sieb. & Zucc. var. *magnificum*
CHENOPODIACEAE
Atriplex patula L.
A. patula L. subsp. *hastata* L.
Beta maritima L.
B. vulgaris L., mangold, sugar beet
Chenopodium amaranticolor Coste & Reyn.
Corispermum hyssopifolium L.

COMBRETACEAE
Combretum erythrophyllum Sond.
C. Kraussii Hochst.

COMPOSITAE
Achillea Millefolium L.
A. Ptarmica L.
A. serbica Petr.
Ageratum Houstonianum Mill.
Andryala varia Lowe
Anthemis arvensis L.
A. Cotula L.
Artemisia Dracunculus L.
A. lactiflora Wall.
Bellis perennis L.
Cassinia Vauvilliersii Hook. f.
Chrysanthemum Parthenium (L.) Bernh.
C. segetum L.
Dimorphotheca Barberiae Harv.
Eupatorium riparium Regel
Hieracium boreale Fries
H. florentinum All.
H. gymnocephalum Griseb.
Humea elegans Sm.
Leptosyne Douglasii DC.
Matricharia Chamomilla L.
M. inodora L.
Pulicaria dysenterica (L.) Bernh.
Tanacetum boreale Fisch.
T. vulgare L.
Tarchonanthus camphoratus L.

CORNACEAE
Cornus canadensis L.
C. glabrata Benth.
C. kousa Buerg.
C. sanguinea L.
C. stolonifera Michx.
Curtisia faginea Ait.
Griselinia littoralis Raoul
G. lucida Forst. f.
Helwingia japonica Dietr.

CRASSULACEAE
Kalanchoe crenata Haw.

CRUCIFERAE
Arabis albida Stev.
A. alpina L.
A. hirsuta (L.) Scop.
A. procurrens Waldst. & Kit.
Aubretia deltoidea DC.
Brassica adpressa Boiss.
B. nigra Koch
B. oleracea L. var. *italica* Plenck (seeds)
B. Rapa L.
B. Sinapis Viscani
Cheiranthus Cheiri L. (seeds)
Crambe fruticosa L.
C. maritima L.
Eruca sativa Mill.
Erysimum linifolium J. Gay (seeds)
Heliophila leptophylla Schlechter
Iberis sempervirens L.
I. umbellata L. (seeds)
Isatis tinctoria L.
Lepidium Draba L.
Lunaria rediviva L.
Mathiola incana R. Br.
Peltaria turkmena Lipsky
Raphanus Raphanistrum L.
R. sativus L. (seeds)
Sinapis alba (L.)
Vella Pseudocytisus L.

CUNONIACEAE
Schizomeria ovata D. Don

DIAPENSIACEAE
Shortia uniflora Maxim. var. *grandiflora* Hort.

DILLENIACEAE
Candollea cuneiformis Labill.
Sladenia celastrifolia Kurz.
DIOSCOREACEAE
Dioscorea balcanica Kosanin
D. villosa L.
Tamus communis L.
DIPSACEAE
Cephalaria alpina Schrad.
EBENACEAE
Diospyros discolor Willd.
D. Ebenum Koen.
Royena lucida L.
ELAEOCARPACEAE
Elaeocarpus cyaneus Sims.
Vallea stipularis L.f.
EPACRIDACEAE
Richea macrocarpa
ERICACEAE
Erica canaliculata Andr.
Gaultheria Hookeri C. B. Clarke
Kalmia angustifolia L.
Leucothoe Davisiae Torr.
Macleania punctata Hook.
Pernettya mucronata Gaudich.
Rhododendron lutescens Franch.
R. megacalyx I. B. Balf. & F. K. Ward
R. ponticum L.
EUCRYPHIACEAE
Eucryphia lucida Druce
EUPHORBIACEAE
Croton Eleuteria Benn.
Euphorbia Hislopii N. E. Br.

FAGACEAE
Castanea sativa Mill.
Quercus Cerris L.
FLACOURTIACEAE
Aberia caffra Hook. f. & Harv.
Hydnocarpus anthelmintica Pierre
H. ilicifolia King
H. Wightiana Blume
Taractogenos Kurzii King
GARRYACEAE
Garrya elliptica Dougl.

GERANIACEAE
Geranium ibericum Cav.
G. neisum
G. viscosissimum Fisch. & Mey.
GRAMINEAE
Arrhenatherum elatius (L.) J. & C. Presl
GUTTIFERAE
Calophyllum Calaba Jacq.

HAMAMELIDACEAE
Bucklandia populnea R. Br.
Disanthus cercidifolius Maxim.
Hamamelis japonica Sieb. & Zucc.
Parrotia Jacquemontiana Decne.
P. persica C. A. Meyer
Sycopsis sinensis Oliv.
Trichocladus grandiflorus Oliv.
HIPPOCASTANACEAE
Aesculus carnea Hayne
A. Hippo-castanum L.
LABIATAE
Clinopodium vulgare L.
Coleus thyrsoideus Baker
Galeobdolon luteum Huds.
Prostanthera Sieberi Benth.
LARDIZABALACEAE
Decaisnea Fargesii Franch.
LAURACEAE
Cryptocarya australis Benth.
Laurus canariensis Webb & Berth.
Lindera sericea Blume
Tetranthera japonica Spreng.
LECYTHIDACEAE
Gustavia speciosa DC.

LEGUMINOSAE
Anthyllis Vulneraria L.
Astragalus massiliensis Lam.
Cassia spectabilis DC.
Ceratonia Siliqua L.
Coronilla varia L.
Derris malaccensis Prain
Medicago lupulina L.
Melilotus altissima Thuill.
Prosopsis jacari Hort.
Sophora tomentosa L.
LILIACEAE
Anthericum liliioides
Asparagus officinalis L. var. *altilis* Kniph.
Aspidistra elatior Blume
Chionodoxa Luciliae Boiss.
C. sardensis Hort.
Chlorophytum sp.
Colchicum autumnale L.
Cordyline stricta Endl.
Dracaena sp.
Fritillaria Meleagris L.
Hyacinthus romanus L.
Lachenalia orchioides Soland.
Muscari botryoides Mill.
Pleomele surculosa N. E. Br.
Scilla Hohenackeri Fisch. & Mey.
S. non-scripta (L.) Hoffmg. & Link
S. siberica Andr.
Trillium grandiflorum Salisb.
Tulipa forsteriana Hort.
T. Kaufmannia Regel
T. Kolpakowskiana Regel
T. linifolia Regel
T. turkestanica Regel
Yucca recurvifolia Salisb.
LOGANIACEAE
Buddleia hybrida
B. Willsii
Chilianthus oleaceus Burch.
LYTHRACEAE
Lafoensia Vandeliana DC.
Lawsonia inermis L.
MAGNOLIACEAE
Drimys aromatica Desc.
D. Winteri Forst.
Magnolia stellata Maxim. var. *rosea* Hort.
Michelia fuscata Blume
MALPIGHIACEAE
Heteropterys umbellata A. Juss.
MALVACEAE
Lagunaria Patersonii G. Don
MARCGRAVIACEAE
Marcgravia umbellata
MELASTOMACEAE
Centradenia grandifolia Endl.
Melastoma corymbosum Sims
MELIACEAE
Entandophragma utile Sprague
Trichilia Dregei E. Mey.
Turraea obtusifolia Hochst.
MELIANTHACEAE
Greyia flannaganii Bolus
G. Sutherlandii Hook. & Harv.
MORACEAE
Dorsteina Barterii Bur.
D. Contrajerva L.
D. elata Gardn.
Ficus macrophylla Desf.
MYRICACEAE
Myrica rubra Sieb. & Zucc.
MYRSINACEAE
Maesa Chisia D. Don
Rapanea salicina Mez.
MYRTACEAE
Angophora lanceolata Cav.
Baeckia virgata Andr.
Callistemon citrinus Stapf var. *splendens* Stapf
C. rigidum R. Br.
Hypocalymna robustum Lindl.
Metrosideros tomentosa A. Rich.
Micromyrtus microphylla Benth.
Myrrhinium atropurpureum Schott
Myrtus obcordata Hook. f.
Psidium Cattleianum Sabine
P. pumilum Vahl
NYCTAGINACEAE
Pisonia Brunoniana Endl.
OCHNACEAE
Ochna atropurpurea DC.
OLEACEAE
Nyctanthes Arbor-tristis L.
Olea capensis L.
O. laurifolia Lam.
Schrebera Saundersiae Harv.
Syringa pekinensis Rupr.
ORCHIDACEAE
Phaius grandiflorus Reichb. f.
OXALIDACEAE
Oxalis Acetosella L.
O. arborea Hort.
O. rubra A. St Hil.
PALMAE
Chamaedorea corallina Hook. f. var. *glaucifolia* H. Wendl.
C. Tepejilote Liebm.
PAPAVERACEAE
Glaucium flavum Crantz. var. *tricolor* Hort.
PASSIFLORACEAE
Passiflora suberosa L.
PHYTOLACCACEAE
Phytolacca acinosa Roxb.
PIPERACEAE
Piper excelsum Forst.
PITTOSPORACEAE
Pittosporum eugenioides A. Gunn.
P. Fairchildi Cheesem.
P. tenuifolium Gaertn.
P. undulatum Vent.
Sollya heterophylla Lindl.
POLEMONIACEAE
Polemonium carneum A. Gray
POLYGALACEAE
Polygala calcarea F. Schultz
P. vulgaris L.
POLYGONACEAE
Eriogonum subalpinum var. *major*
Polygonum Auberti L. Henry
P. paniculatum Bl.
Rumex conglomeratus Murr.
R. obtusifolius L.
R. sanguineus L.
Ruprechtia corylifolia Griseb.
PORTULACACEAE
Lewisia cotyledon B. L. Robs.
L. Tweedyi B. L. Robs.
PRIMULACEAE
Anagallis arvensis L.
Cyclamen persicum Mill.
Dodecatheon pauciflorum Greene
Hottonia palustris L.
Lysimachia Barystachys Bunge
L. clethroides Duby
L. nemorum L.
L. Nummularia L.
L. vulgaris L.
Primula denticulata Sm.
P. floribunda Wall. var. *Isabellina* Hort.
P. florida Balf. & Forr.
P. japonica A. Gray
P. kewensis W. Wats.
P. lichiangensis Forr.
P. pulverulenta Duthie
P. veris L.
P. verticillata Forsk.
P. vulgaris Huds.
Steironema ciliatum Rafin.
PROTEACEAE
Knightia excelsa R. Br.
RANUNCULACEAE
Anemone blanda Schott & Kotschy var. *scythinica* Hort.
A. nemorosa L.
A. ranunculoides L.
Caltha palustris L.
Cimifuga japonica Spreng.
Nigella damascena L.
Paeonia Emodi Wall.
P. humulus var. *villosa*
P. lutea Franch.
P. mollis Anders.
Ranunculus arvensis L.
R. Ficaria L.
R. trichophyllus Chaix
RESEDACEAE
Reseda lutea L.
RHAMNACEAE
Discaria serratifolia Benth. & Hook.
D. toumatou Raoul
Pomaderris microphyllum
ROSACEAE
Agrimonia Eupatoria L.
Filipendula ulmaria (L.) Maxim.
Prunus Laurocerasus L.
Pyrus serrulata Rehd.
P. ussuriensis Maxim.
Spiraea nipponica Maxim.
S. Thunbergii Sieb. & Zucc.
RUBIACEAE
Gardenia jasminoides Ellis
G. Thunbergia L.f.
Ixora macrothyrsa Teysm. & Dinn.
Pavetta lanceolata Eckl.
P. obovata E. Mey.
Sarcocephalus Russeggeri Kotschy
RUTACEAE
Boenninghausenia albiflora Reichb.
Casimiroa edulis La Llave
Evodia elegans var. *rudleyi*
Murraya exotica L.
SALICACEAE
Populus alba L.
Salix fragilis L.
S. purpurea L.
SAPINDACEAE
Paullinia barbadensis Jacq.
SAPOTACEAE
Achras Sapota L.
Chrysophyllum Cainito L.
Dichopsis obovata C. B. Clarke
Lucuma mammosa Gaertn. f.
Mimusops elengi L.
Sideroxylon inerme L.
SAXIFRAGACEAE
Bauera rubioides Andr.
Bergenia ligulata Engl. var. *speciosa*
B. pauciflora
B. speciosa
Ribes cereum Dougl.
R. laurifolium Jancz.
Saxifraga apiculata Engl.
S. apiculata Engl. var. *alba* Hort.
S. cotyledon L.
S. crassifolia L.f.
S. trifurcata Schrad.
S. umbrosa L.
S. vandellii Sternb.
Schizophragma integrifolia Oliv.
SCROPHULARIACEAE
Campylanthus salsoloides Roth.
Celsia Arcturus Jacq.
Digitalis purpurea L.
Melampyrum pratense L. var. *latifolium* Syme
Scrophularia aquatica L.
S. nodosa L.
SIMARUBACEAE
Picraena excelsa Lindl.
SOLANACEAE
Browallia speciosa Hook. var. *major* Hort.
Capsicum annuum L.
Cestrum aurantiacum Lindl.
C. Newelli Hort.
C. psittacinum Stapf
C. roseum H. B. K.
C. scaber
C. Smithii Hort.
Hyoscyamus niger L.
Lycopersicum esculentum Mill.
Nicotiana rustica L.
Solanum capsicastrum Link
S. Dulcamara L.
S. nigrum L.
S. tuberosum L. var. *Catriona*

STACHYURACEAE
Stachyurus praecox Sieb. & Zucc.
STERCULIACEAE
Hermannia colorata
STYRACACEAE
Halesia carolina L. var. *Meehanii* Perkins
TACCACEAE
Tacca artocarpifolia Seem.
THEACEAE
Camellia reticulata Lindl.
C. Sasanqua Thunb.
Gordonia anomala Spreng.
G. excelsa Blume
Ternstroemia japonica Thunb.
Visnea Mocanera L.f.
THEOPHRASTACEAE
Deherainia smaragdina Decne.
THYMELEACEAE
Dais cotinifolia L.
TROCHODENDRACEAE
Euptelea Davidiana Baill.
UMBELLIFERAE
Aethusa cynapium L.
A. cynapium L. var. *agrestis* Wallr.
Apium graveolens L. var. *dulce* DC.
Caucalis Anthriscus Huds.
Chaerophyllum nodosum Lam.
Heracleum persicum Desf.
Ligusticum scoticum L.
Pastinaca sativa L.
Pimpinella Saxifraga L.
Sanicula europaea L.
Smyrnium olusatrum L.
URTICACEAE
Debregeasia longifolia Wedd.
VALERIANACEAE
Centranthus ruber DC. var. *albus*
Valeriana dioica L.
V. officinalis L.
V. Phu L.
V. pyrenaica L.
VERBENACEAE
Citharexylum Pringlei Greenm.
Duranta glauca
Gmelina Hystrix Schultes
VIOLACEAE
Hymenanthera Traversii J. Buchan
VITACEAE
Leea angulata Korth.
ZINGIBERACEAE
Globba winittii C. H. Wright
ZYGOPHYLLACEAE
Porlieria hygrometra Ruiz & Pav.
GNETALES
Gnetum Gnemon L.
PTERIDOPHYTES
Selaginella Braunii Baker
S. canaliculata Baker
S. Wallichii Spring.
S. Wildenowii Baker
AGARICACEAE
Entoloma prunuloides Fr.
E. sinuatum Fr.
Hygrophorus niveus (Scop.) Fr.
Lactarius piperatus (Scop.) Fr.
Naucoria temulenta Fr.

Tolerance of the conidia to hydrogen-ion concentration

Conidia incubated in phosphate buffers over a range from *p*H 4·5 to 9·0 gave 75–97% germination at *p*H 4·5, 6·0, 6·8 and 8·0. There was no germination at *p*H 9·0. Many plant extracts fall within the range *p*H 4·5–8·0, the exceptions being more acid. Acetate buffers were used to try to determine the germination in more acid conditions, but since the acetate ions appear to reduce germination at any hydrogen-ion concentration the experiments were unsuccessful.

Dry-plant material

Samples of twenty-four active plant species were left to dry at room-temperature and were tested at intervals for activity. The two members of the Caprifoliaceae, *Lonicera Periclymenum* and *Symphoricarpos albus*, and the two members of the Umbelliferae, *Pimpinella Saxifraga* and *Pastinaca sativa*, so tested lost their activity in from 2 days to 2 weeks. In the Cruciferae, the activity of *Raphanus Raphanistrum* was lost after two weeks of drying, while that of *Brassica Sinapis* was retained for at least 2 months. In the Compositae, *Hieracium boreale* lost all activity after 3 days drying, while *Chrysanthemum segetum* and *Bellis perennis* were still active after 3 months and 9 months respectively. Two representatives of the Ranunculaceae were dried, *Anemone nemorosa* and *Ranunculus Ficaria*, and both were active for at least 2 months. *Atriplex patula*, *Clinopodium vulgare* and *Cornus sanguinea* were all active after 8 months of drying.

Active plants tested for fungicidal or fungistatic action

Apparently largely fungistatic	Apparently fungicidal	
Cornus sanguinea	*Anemone nemorosa*	*Pimpinella Saxifraga*
Ranunculus Ficaria	*Allium cepa*	*Primula vulgaris*
Chrysanthemum segetum	*Atriplex patula*	*Salix purpurea*
Raphanus Raphanistrum	*Clinopodium vulgare*	*Symphoricarpos albus*
	Hedera helix	

Dilution tests

Some of the active extracts from readily obtainable plants were serially diluted, generally to 1 in 32, with glass-distilled water, and the dilutions were tested in the same way. The table shows the highest dilution at which inhibition of germination was complete:

Full strength	$\frac{1}{2}$ strength	$\frac{1}{4}$ strength	$\frac{1}{16}$ strength
Brassica spp.	*Allium cepa*	*Atriplex patula*	*Caltha palustris*
Chrysanthemum segetum	*Clinopodium vulgare*	*Bellis perennis*	*Primula vulgaris*
Medicago lupulina	*Castanea sativa*	*Cornus sanguinea*	
Raphanus Raphanistrum	*Ranunculus Ficaria*	*Pimpinella Saxifraga*	
Solanum dulcamara	*Salix purpurea*	*Scilla nutans*	
Symphoricarpos albus	*Tamus communis*		
		$\frac{1}{32}$ strength	$\frac{1}{128}$ strength
		Anemone nemorosa	*Hedera Helix*
		Scrophularia nodosa	

Hedera Helix (Ivy) was entirely exceptional in giving complete inhibition at a dilution of 1 in 128. Extracts from leaves, flowers, fruits and seeds were all active. Leaf extract retained activity after 2 hr. boiling, but an extract made from leaves steamed for 10 min. was almost completely inactive. Leaves dried at room temperature retained their activity for at least 3 months.

Distribution of inhibitors within the plants

Parts of some of the commoner active plants were tested separately, and except in some Cruciferae, of which only the seeds showed activity, the leaves were always active. Usually flowers were active too, fruits less frequently. Roots were rarely available, but were active in *Ranunculus Ficaria* and mangold.

In the Salicaceae, *Salix fragilis* and *S. purpurea* had active young leaves and inactive mature leaves, bark, flowers and roots. In the Caprifoliaceae *Lonicera Periclymenum* had active leaves only, and these were inactivated when ground with flowers. On the other hand, *Viburnum lantana* and *V. opulus* both had inactive leaves and flowers and active fruits. In the Solanaceae *Solanum Lycopersicum* and *S. dulcamara* had active leaves and inactive fruits. *S. tuberosum* had active leaves and inactive tubers.

The inhibitory action of these *Solanum* spp. may be correlated with the solanin content of the various parts of the plants, since the fruits of *S. Lycopersicum* and the tubers of *S. tuberosum* contain no solanin and are inactive, whereas the solanin-containing foliage is active.

Distribution of inhibitors in time

Aesculus Hippocastanum and *Castanea sativa*, which had active leaves throughout the summer, gave in the autumn inactive extracts from yellowing leaves. Conversely, early in the spring these two trees and *Acer pseudo-platanus*, *Quercus cerris*,

and *Cornus sanguinea* gave inactive or partially active extracts from the leaves, but by May full leaf activity had developed in most cases.

This suggests that the greater susceptibility of young tissues to some plant pathogens, generally attributed to the poor development of their mechanical protection, e.g. cuticle, may occasionally be due to the late development of chemical protection.

Primulaceae

This was the most constantly active family, each one of the twenty-two species tested giving completely inhibitory extracts. The extract from primrose leaves was completely inhibitory at a concentration of 1 in 16, extracts boiled for 2 hr. retained full activity and extracts made from leaves steamed for 2 hr. were active. The inhibitory action is probably fungicidal.

Rosaceae

Eighty-one species were tested and only seven gave inhibitory extracts, two of them being produced by leaves and flowers ground together of *Pyrus serrulata* and *P. ussuriensis*. The leaves of seven varieties of apples were tested and all gave inactive extracts, regardless of whether they were alleged to be susceptible or resistant to apple scab.

Work done by Wiltshire (1915) and by Johnstone (1931) showed that there was a correlation between varietal resistance and reduction of conidial germination in expressed leaf saps, but that age of tree, age of foliage, age of fruits and nitrogen content of the tree all affected the inhibitory powers of the liquids. Johnstone concluded that there was probably a disparity between expressed sap and epidermal sap, and that chemical resistance to apple scab was located in the latter. It is therefore all the more noteworthy that two *Pyrus* spp. were found to give inhibitory extracts.

This work was carried out during the tenure of a grant from the Agricultural Research Council, and the author wishes also to thank Dr H. B. S. Montgomery of East Malling Research Station for his help with his testing technique, and Professor F. T. Brooks for his advice and criticism in the preparation of the paper.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held on Friday, 4 October 1946, in the Imperial College of Science and Technology, London; the President, Dr C. B. Williams, in the Chair.

The significance of trace elements in plants and animals

The following papers were read and discussed:

1. General introduction. By Sir Edward J. Salisbury.
2. Visual diagnosis of mineral deficiencies in plants. By Prof. T. Wallace.
3. Chemical tissue tests. By Mr D. J. D. Nicholas.
4. The use of leaf analysis, plant injection, and curative treatment for the determination of mineral deficiency in plants. By Dr W. A. Roach.
5. Trace elements in relation to enzymes. By Dr R. Hill.
6. Trace elements in relation to health of livestock. By Dr H. H. Green.

GENERAL INTRODUCTION

By Sir Edward J. Salisbury

The subject of the significance of trace elements which is to be discussed to-day concerns alike those of plants and animals. The precise roles that each nutrient plays in the physiology of plant or animal are clearly amongst the most important problems that the future holds for us. It is not the least fruitful aspect of such research that it will involve the integrated effort of the plant and animal ecologist, the physiologist, the chemist and the physicist. For in these days of increasing specialization any field of study that tends to emphasize the fundamental unity of all knowledge is a contribution not to itself alone but to the advance of that real education that is wisdom.

It is, I conceive, worth while asking ourselves what we mean by nutritional deficiency. In general the term tends to be applied where there is obvious loss of vigour or diminished yield associated with the insufficiency or absence of a particular nutrient. But it would be a rash man who would undertake to define in measurable terms a healthy plant or a healthy animal; hence we resort to 'yardsticks' that are at best rather crude. We all recognize the relativity principle but perhaps too frequently forget that limiting factors are of the most diverse kinds. If a plant grows better under a given set of conditions when we supply added manganese, we say the organism suffers from manganese deficiency, but although we know that a cereal in the field is suffering from severe competition we do not commonly recognize that its nutrition is suffering from space deficiency.

Few fields of biological study can vie in both scientific interest and practical importance with that of nutrition, for on the one hand it involves fundamental knowledge of the physiological ecology of the organisms concerned, the fascination of which has in recent years been enhanced by the recognition of the role of the vitamins in the organic domain and of the trace elements in the inorganic. The study of the part played alike by vitamins and trace

elements has brought home to us something of the complexity of the nutritional problem and the realization of how much we can owe to how little. When I was a student nearly forty years ago in the heyday of the worship of calories it would I think have been regarded as inconceivable that the presence or absence of about one part of copper in forty millions could differentiate between the death and healthy survival of animals even had there been methods then available for determining within reasonable accuracy amounts of such diminitude. The fact is that even for members of our own species our yardsticks for measuring health are in the scientific sense crude, for other animals and plants they are even cruder. Moreover, because the organisms we use for purposes of study are often of economic importance there is real danger of confusion of thought between what is economically desirable and what, scientifically considered, is beneficial to the organism. Such confusion of thought might, even from the economic aspect, lead to temporary gains at the expense of long term yields.

Nutritional deficiences can I think be usefully classified into (i) real deficiencies and (ii) conditioned deficiencies. By the former we understand either complete absence of a nutrient or its presence in too small an amount to meet the needs of a given organism. Deficiency diseases due to such causes can manifestly be alone remedied by the supply of the missing nutrient in available form. The conditioned deficiencies, however, where the nutrient is neither absent nor present in amounts too small to be effective yet is not adequately available, are subject to remedial action as varied as the conditioning factors that bring about the non-availability. The conditioning factors that we are as yet most familiar with are chemical ones such as the natural deficiency of manganese on calcareous soils. The apparently complex relations between the absorption of calcium and boron and the ratio effects of various trace elements are special examples of such chemical factors. How important are the physiological conditioning factors will only be apprehended as we learn more concerning the function of the various nutrients. The importance of biological factors in connexion with the availability of manganese in the soil has already been demonstrated by Quastel and his associates. The importance of cobalt for ruminants as demonstrated by Marston is another pertinent example. The extremely diverse capacity of different species to obtain trace elements from the same soil may be physiological or biological or both. That the Rubiaceous plant *Faramea insignis* can accumulate aluminium to the extent of 40 parts per thousand or nearly 200 times the amount in other species is doubtless largely physiological, just as the need of and high accumulating capacity for selenium of *Astragalus bisulcata* is obviously an intrinsic characteristic. But the greater susceptibility of apples on dwarfing stocks to some deficiency diseases as compared with the same variety on a non-dwarfing stock is perhaps conditioned by the morphological factor of the more extensive root system of the one as compared with the other, with consequent increase in the volume of soil exploited. Competition for trace elements between organisms and between organs is another biological aspect that as yet has only been touched on. That organisms by their accumulative action can enrich the surface layer of the soil is a feature to which attention has been drawn in various connexions, but how far the accumulating species assists or retards availability for other organisms is almost an untouched field that would well repay investigation as also the physiological characteristics that differentiate susceptible from non-susceptible organisms.

The work of Keilin and others has done much to further our knowledge of the role of trace elements in animals but in this direction plant physiology lags behind. It is clear that the problems before us involve diagnosticians, pathologists, ecologists, as well as workers in pure science, and if the complex relations add to our difficulties they also add to our interest. Indeed perhaps the only safe criterion of progress is a growing apprehension of ignorance. It is therefore both as an adventure of the human intellect no less than as a contribution to the solution of practical problems that I commend a discussion which I am sure will be the more profitable because of the different angles from which it will be approached by the different speakers.

VISUAL DIAGNOSIS OF MINERAL DEFICIENCIES OF PLANTS

By T. Wallace, *Long Ashton Research Station, Bristol*

In introducing this subject to-day there are two points to emphasize concerning the various methods for determining mineral deficiencies in crop plants. The first is that no one of these alone will give a complete answer to all problems of mineral nutrition though together I believe they are capable of solving any problem involving deficiencies or excesses of any of the known mineral nutrients of plants. The second is that the methods are most effective when used in complementary and confirmatory roles.

In practice it is not, of course, necessary to use every method for each problem: one method will sometimes suffice and more often a particular investigator will use his own favoured methods or the most convenient methods according to the circumstances. For example at Long Ashton we commonly use a combination of the visual and tissue test methods for advisory problems and for following the progress of nutrient treatments in field experiments.

Viewed against this background I regard the visual method as an excellent method of approaching field problems of mineral nutrition and one which, in many instances, is capable of detecting the main deficiencies affecting growing crops or likely to affect particular crops which may be planted subsequently on a given site.

The basis of the visual method

The applicability of this method for diagnostic purposes depends on the fact that plants deficient in any mineral nutrient develop well defined and characteristic signs in various organs, particularly in the leaves. These signs generally differ on any given plant for the various nutrients, whilst the effects produced over a wide range of plants for a deficiency of any one element often show similar features. Although on first introduction to the various visual effects they may appear heterogeneous, they do, in fact, allow of a considerable degree of grouping. Thus for deficiencies of nitrogen, phosphorus, potassium and magnesium the older parts of the plants are generally first affected, whilst for shortages of calcium, iron and boron, deficiency effects begin at growing points.

It must be stressed that before the visual method can be used easily and effectively it is necessary to become familiar with the variations which may result from any given deficiency in different plants.

The field problem is often much simplified by the presence of indicator plants. These are plants which show special susceptibility and very distinctive effects for specific deficiencies.

To provide a basis for using the visual method it is necessary first to produce the visual effects in the various crops and to record the results for reference. This is done by growing plants under controlled conditions of nutrient supply, usually in sand cultures, to produce the various effects and subsequently checking the results under field conditions. The visual effects can be recorded as colour photographs, the negatives of which can be preserved without appreciable deterioration for several years.

Use in the field

As a preliminary to using the visual method in the field it is often valuable to note certain aspects of site and soil and to examine the crop for obvious signs of damage from pests and diseases.

As regards soil conditions, texture, drainage, *p*H, dryness and cultural conditions are often helpful in ruling out certain deficiencies and in pointing to others. The presence of certain pests and diseases may complicate the symptoms and in extreme cases may even rule out the use of the method.

So far as the visual method is concerned mineral deficiencies in the field may be classified into both simple and complicated categories. In the former group it is possible to recognize the deficiency immediately by decisive symptoms on the crop. Examples occur when the crop is an indicator plant for the particular deficiency or when an indicator plant is growing nearby and can be used to confirm the diagnosis. Thus potassium deficiency can be determined accurately in potatoes, manganese deficiency in oats and boron deficiency in sugar beet, whilst a doubtful case of potassium or magnesium deficiency in apples can be decided if a gooseberry or blackcurrant crop is available for confirmation.

Indicator plots. The visual method can be applied in conjunction with special indicator plots to forecast the likelihood of mineral deficiencies on soils of unknown cropping potentialities. This procedure has been used during the war on difficult areas brought under cultivation in the ploughing-up campaign.

In these plots indicator plants for the various deficiencies are sown in strips crossed by a series of different fertilizer and spray treatments which ensure that all crops are growing under 'treated' and 'untreated' conditions with regard to each of the mineral nutrients. This layout not only tests highly susceptible crops under possible deficiency conditions but also shows the effects of supplying to a number of crops the various elements which may be deficient.

Special uses of the visual method

Two special virtues of the visual method are speed in operation and the fact that no equipment, save occasionally a soil indicator outfit for *p*H and an auger, is necessary. These attributes make the method particularly suitable for survey work and it has been extensively used by Dr Plant for this purpose in various areas of the country during the war period. The method, with certain exceptions, is best applied to broad-leaved crops and is of special use for long-term fruit crops for which fertilizer responses may be very slow. The visual method, in fact, provided the clues to the potassium and magnesium deficiency problems in fruit trees and also suggested that the important factor in 'grass effect' on fruit trees was nitrogen deficiency.

More recently the method has been applied to the resolution of factors concerned in the soil acidity complex. Hitherto it had been shown that there are several possible reasons why plants should fail on acid soils, but which was causal in particular cases had not been determined with certainty.

Using the visual method in sand culture experiments, it has been possible to examine the effects of such factors as H-ion concentration, calcium deficiency, manganese toxicity and aluminium toxicity on a number of crop plants and to show which of the factors was responsible for failure symptoms observed in the field.

Thus in runner beans excess manganese produces the typical failure effects; in potatoes and cauliflowers calcium deficiency and manganese toxicity are both concerned; whilst for sugar beet *p*H, manganese toxicity and calcium deficiency may all be concerned but each may not be of the same importance in all instances. In correcting soil acidity the visual method, used on mangolds and sugar beet, has shown that the use of magnesian lime treatments on certain soils, whilst correcting soil acidity and making cropping possible, has nevertheless resulted in crops deficient in calcium.

Conclusion

In concluding this paper I should like to emphasize that the visual method of diagnosis is not a hit and miss method to be used by those who are not prepared to study their crop plants in detail. Skill in its use can only be acquired by intensive study and experience.

Finally may I express the hope that future generations of plant pathologists will receive some training in methods of diagnosing the mineral status of plants and that in any such training the visual method will not be neglected.

CHEMICAL TISSUE TESTS

By D. J. D. Nicholas, *Long Ashton Research Station, Bristol*

In recent years interest has been focused on the use of rapid chemical tests to assess the mineral status of soils and plants. Some pioneer work on the application of quick tests to soil extracts and plant tissues has been carried out by a number of American workers. Hoffer (1926) tested fresh corn stalks for nitrates and potassium and later Emmert (1934) showed that tests for phosphate and nitrate in the conducting tissue of tomato and lettuce could be used as indicators of fertilizer needs. These quick tests were used by Carolus (1938) and were later improved by Thornton *et al.* (1939) who published the Purdue tissue testing system mainly for the evaluation of potassium, phosphate and nitrate. Further contributions have been made by many workers including Hance (1941), Hester (1941), Page & Burkhart (1941) and Scarseth (1943). Among the notable contributions to the quick testing of soil extracts are those of Hester (1934) and Morgan (1936). The latter's Universal soil testing system has been used in America and in this country.

Work on chemical tissue tests was commenced at Long Ashton in 1943. The existing methods were examined and a technique has been devised to test a number of the easily soluble nutrients in fresh plant material (Plant *et al.* 1944; Nicholas & Jones, 1944, Nicholas, 1945). The method has been successfully used in the diagnosis of mineral deficiencies and toxicities in crop plants and has served as an invaluable confirmatory test to the visual method (Wallace, 1943).

The extensive use of visual diagnosis as a method for the identification of mineral deficiencies has emphasized the need for a quick tissue testing system to confirm the visual technique. These chemical tests are of special value when visual symptoms may be ill-defined or complicated by other factors, e.g. drought, frost, fungi, insects, virus and a complex of nutrient disorders.

Definition

Tissue testing entails the extraction of the easily soluble nutrients from fresh plant material and the subsequent testing of the extracts chemically. Its value as a diagnostic method is based on the fact that the extractable nutrients from healthy compared with those from mineral deficient plants usually show significant differences for the limiting mineral element. Toxicity levels of nutrients can also be detected by comparison with normal plant extracts. It is essentially a semiquantitative method that can be readily used in the field with the minimum of time and apparatus.

Technique of chemical tissue tests

Field sampling methods

In sampling fresh plant material for chemical tissue tests the following procedure is adopted. Leaf samples are taken from fixed positions so that they are comparable in size, maturity and probably in metabolic activity. This is important as there is usually a nutrient gradient in crop plants, e.g. higher K content in younger as compared with older leaves and the reverse for Ca. Positions for sampling various crops are as follows:

Potato, tomato and hop. Leaves from the mid-stem position.

Cereals. The internode adjacent to the growing point. At the very young stage the first formed leaf may be used.

Onion. The leaf bases.

Cauliflower, sugar beet, mangold, etc. The first upright leaf position.

Fruit trees. Leaves from the base of the new leader growths.

When collecting material for making comparisons between experimental plots, random leaf samples are taken; for diagnosis in the field comparisons are made between good and poor specimens.

Preparation of test samples

In most instances the stalk or petiole portion is taken in preference to the lamina as the pigment concentration in the former is usually less whereas the nutrient conducting tissue is greater. Both tissue test and complete chemical data agree in showing that petiole material reflects the nutritional status of the plant as efficiently as the leaf lamina. In testing Brassicae the midrib portions of comparable girth are used.

The stalk portions are weighed ($\frac{1}{2}$ g. per test) and then cut up into approximately 2 mm. disks on a tile using a sharp scalpel.

Extraction

For the extraction of the easily soluble nutrients from plants, two solutions are recommended for use:

1. *Morgan's reagent* (glacial acetic acid buffered by means of sodium acetate to a *p*H of 4·8). This is used for the extraction of K, Mg, Ca, PO_4, NO_3, Cl and Mn, the latter when present in excess.

For the detection of Mn deficiency levels a purified Morgan's solution is made up involving redistillation of the acetic acid and recrystallization of sodium acetate.

2. *Hydrochloric acid* (conc.). Used for the extraction of Fe and Zn, the latter when present at toxic concentrations.

The following units are adopted: 0·5 g. of macerated fresh tissue is steeped 15 min. in 5 ml. of Morgan's reagent. The time is important and must be kept constant as maximum differences are obtained between healthy and deficient plants only after that interval.

When a series of tests is contemplated, bulk extracts are made using 3 g. of material soaked 15 min. in 30 ml. of the buffered solution. Then the extract is filtered through cotton-wool into a specimen tube. 5 ml. is transferred to other clean flat-bottomed tubes. The extracts are now ready for chemical testing. It will be noted that tissues absorb a little of the buffered solution, hence five lots of 5 ml. can be obtained from the original 30 ml. added.

Zn and Fe extraction. 1 ml. of HCl is added to 0·5 g. of macerated tissue and allowed to stand for 15 min. Then 5 ml. of distilled water is added and, after shaking, the extract is pipetted off into a clean specimen tube and is now ready for testing.

Chemical tests used

Details of the chemical tests used have already been published (Nicholas & Jones, 1944) and will be only briefly mentioned here:

Nutrient tested	Reagents
Potassium	Sodium cobaltinitrite and isopropyl alcohol
Calcium	Ammonium oxalate
Magnesium	Titan yellow, hydroxylamine hydrochloride, sucrose and caustic soda
Manganese (present in excess)	Formaldoxime and caustic soda
Manganese (at deficiency levels)	Tetramethyldiaminodiphenylmethane and potassium periodate
Ferric iron	Potassium thiocyanate
Zinc (present in excess)	Cobalt acetate, potassium thiocyanate and mercuric chloride
Nitrate	Phenoldisulphonic acid and ammonia
Phosphate	Ammonium molybdate, hydroquinone and sodium carbonate and sodium sulphite
Chloride	Silver nitrate

The tetramethyl test can detect minute amounts of manganese in the tissue extracts, its sensitivity range being 100 parts to 1 part per 1000 million whereas the formaldoxime test

can only detect 200 parts per 1000 million. Manganese deficiency can be readily confirmed in a number of crop plants and the test is especially useful in cereals where the visual signs can be difficult to detect. This test, however, cannot be used in the field as it is essential to have rigidly controlled conditions which are only available in a laboratory.

Zinc toxicity has been reported from areas in the Mendip country of Somerset, from parts of North Wales where zinc deposits occur naturally in the soil, from certain areas adjacent to industrial plants and where untreated industrial sewage has been used on arable land. A high iron tissue value in the affected specimens confirms zinc poisoning as the latter is conducive to the accumulation and the immobilization of iron in the tissues. The affected specimens usually exhibit visual signs of iron deficiency as the result of the unavailability of the iron within the plants.

A Long Ashton Tissue Test Outfit has been designed in conjunction with Messrs Tintometer, Salisbury. This is a useful portable set that can be used in the field.

Nutrient standards

The assessment of turbidity or colour is made initially by eye; the following broad categories are adopted: High, Medium and Low. The use of a plus and minus sign with each is used to subdivide these values. In all instances a quantitative determination is made of the particular cation or anion present in the extract. A Lovibond comparator and disks are used for phosphate, nitrate and magnesium, but prepared standards are made up for potassium, calcium, chloride, manganese (at toxic and deficiency levels) and zinc (present in excess). The values for the high to low range vary with (1) type of nutrient, (2) crop plant. Nutrient standards have been fixed for a range of agricultural and horticultural plants and it must be emphasized that a working knowledge of these is essential for accurate diagnostic work. The medium minus to the low plus range is regarded as the threshold level for a given nutrient usually accompanied by the onset of pathological symptoms of the deficiency.

High levels of manganese are usually recorded in tissue extracts from susceptible plants grown under sharply acid conditions. Excess chloride can readily be detected in a number of crop plants, e.g. potato, when heavy dosages of potassium or sodium chloride have been incorporated in the fertilizer programme.

At the High + + manganese level typical 'acidity' symptoms are usually noted in susceptible crops, e.g. potato and brassica, and the calcium level in such plants is usually low. Chloride damage as evidenced by a brown edge scorch can accompany the High + + chloride level in tissue extracts.

Diagnostic value

Within a short time a confirmation of the visual diagnosis can be obtained using the described tissue test technique. Straightforward deficiencies of potassium, magnesium, phosphorus, nitrogen, calcium and manganese as well as toxicity levels of manganese, chloride and zinc can be readily confirmed. The inter-relations of the soluble nutrients can also be studied, e.g. high manganese and low calcium; high potash and low nitrogen levels can depress magnesium; low potash may lead to higher calcium, magnesium, nitrate and phosphate values.

Tissue test results often give a clue to an impending mineral deficiency prior to the development of the starvation symptoms. This provides in some instances an opportunity to remedy a deficiency before the onset of the visual symptoms. A knowledge of the seasonal nutrient cycle in crop plants is essential for early diagnostic work, so that the values obtained can be related to the seasonal trend. The following example will illustrate this point; a medium tissue test value for potassium obtained in potato during early May is usually serious as it heralds the approach of potassium deficiency, whereas a similar value in late August can be the natural outcome of the downward seasonal trend. An intensive study has been made of the seasonal nutrient cycles in a number of crop plants under various nutrient treatments

to determine at every growth stage a mineral deficiency effect as distinct from a seasonal effect.

The tissue test method should also be of value in ecological studies and in determining the distribution and translocation of nutrients in various plant organs.

Limitations of tissue tests

When a mineral element is deficient in plants, lower tissue test values are usually recorded for the particular nutrient, as compared with those of healthy specimens. Iron does not conform to this rule. This is due to the fact that true iron deficiency is seldom operative under field conditions, but is rather induced by other nutrients, e.g. high lime, or in special instances by high zinc or copper. This results in the immobilization of iron in the tissue and although there is usually abundant iron present, it is not 'active' and hence visual signs of the deficiency are produced. The tissue test for iron may be used to confirm zinc toxicity in plants, as such material usually contain higher amounts of iron than healthy specimens.

The nitrate test can be used as an indicator of the nitrogen status of most crop plants only during the early growth stage. As the plants mature the nitrate seasonal cycle usually falls abruptly, and from mid to late season there is little nitrate present in plant tissues. There appears to be a correlation between wet conditions, probably inhibiting the activity of the nitrifying soil bacteria, and low nitrate values within the plant. Should nitrogen deficiency occur a diagnosis can be made by tissue test early in the season.

It has not been possible to adopt a chemical means for the detection of boron in tissue extracts. This is partly due to the fact that the known methods are not sufficiently sensitive. Most of them require a refined technique involving the use of a 98% sulphuric acid for the development of the boron-quinone coloured complexes.

Correlation with other diagnostic methods

In assessing the value and reliability of the tissue test method it is essential to correlate the data obtained with those of other diagnostic methods, namely (*a*) visual method, (*b*) spray or injection of plants, (*c*) full chemical analysis of healthy as compared with affected specimens. Soil analysis can also give support to the above methods especially in potash, phosphate and lime deficiencies.

Visual diagnosis

In practically all instances there is a close correlation between tissue test results and the visual signs of a nutrient deficiency or toxicity. Threshold values have been fixed which usually coincide with the appearance of the pathological symptoms. Tissue test results often give a clue to an impending nutrient deficiency before the onset of the visual symptoms.

The exceptions have already been mentioned, viz. (1) iron deficiency, and (2) nitrogen deficiency late in the season. In these instances the visual method is more reliable.

Full chemical analysis of the leaves

This is a recognized diagnostic method which involves a chemical comparison between the total nutrient content of healthy plants and that of deficient plants, when in most instances significant differences are noted for the limiting nutrient (iron is an exception). The tissue results for the 'soluble' unassimilated nutrients usually agree with the full chemical data, the latter representing the total nutrient content. In most instances there is a linear relation between the two chemical methods.

Soil data

In general, tissue test results for plants growing on well established manurial plots reflect fertilizer treatment and agree with the soil analysis. Survey work shows, however, that the correlation is not always good. Anomalies have been noted in that soil chemical figures for

potash or phosphate can be adequate but tissue test values have recorded deficiency levels and in most instances visual signs of the deficiency appear. In this instance, soil factors such as aeration, waterlogging or consolidation may affect the uptake of nutrients. Other cases have been noted of soils with low available potash, e.g. 0·005 % K_2O, supporting a susceptible crop such as potato which showed an adequate potassium tissue value and no visual signs of deficiency. This may well be due to the potash although low being readily available to the plants.

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THE USE OF LEAF ANALYSIS, PLANT INJECTION AND CURATIVE TREATMENT FOR THE DETERMINATION OF MINERAL DEFICIENCY IN PLANTS

By W. A. Roach, *East Malling Research Station, Kent*

This contribution is concerned largely with some of the work of the East Malling Mineral Deficiency Team.* The team was enlarged by the Agricultural Research Council with the backing of the Agricultural Improvement Council early in the war to extend to agricultural crops diagnostic methods evolved primarily for fruit. The late Dr Topley and Sir Edward Salisbury did much to bring this about.

Diagnostic methods

This team has concentrated mainly, but not exclusively, on three methods for diagnosing mineral deficiency:

1. Leaf analysis.
2. Plant injection.
3. Measurement of the effect of curative treatment on yield and quality of crop.

The first two are used only to give a preliminary diagnosis—preliminary because the practical value of a diagnosis can only be measured by the extent to which it leads to a complete cure. Reasons will be given later for looking on this third method, for the time being, at least, as an essential part of almost every diagnosis.

It will be convenient to refer briefly to leaf analysis and plant injection before considering the results obtained from a combination of all three methods.

Leaf analysis for our present purpose must determine not only nitrogen, phosphorus, potassium, calcium and magnesium but also traces of iron, manganese, zinc, boron, copper, nickel and probably other elements such as molybdenum. The determination of these traces in the presence of relatively very large amounts of phosphorus, potassium and calcium presents really difficult analytical problems, especially if accurate quantitative figures are desired. From this point of view there is room for improvement in the methods used at present. A physical chemist and physicist have recently been appointed for this work. But for most diagnostic purposes quantitative figures are unnecessary. A variety of methods are in use, only some of which are strictly quantitative:

(*a*) Colorimetric, for phosphorus, boron, copper and iron.

(*b*) Flame spectrographic, for potassium, calcium, magnesium and manganese.

(*c*) Polarographic: this will at present determine excesses of zinc but it is hoped that modifications will enable it to detect deficiencies of zinc and other elements.

Many difficulties are avoided if diagnoses are always based on comparisons of samples from commercially unsatisfactory plants with comparable samples from satisfactory plants.

When a sample contains less of any element than is contained in a sample from a crop known to be borderline in this respect it may be concluded that a perfect crop will not be obtained until the deficiency is made good. But conversely it is not safe to assume that making good the deficiency diagnosed will necessarily bring the crop up to the optimum, because there may be another undetected mineral, or other, deficiency.

* The team consisted of W. A. Roach, S. G. Thompson, and W. O. Roberts, of the East Malling Research Station; B. D. Bolas and D. W. Goodall of the Research Institute of Plant Pathology, Imperial College of Science and Technology; and the following workers appointed by the Agricultural Research Council: J. P. Riches, J. G. Maynard, A. T. Legg, C. Barclay and H. M. Holmes. Some of the work has already been published in detail. The remainder is being prepared for publication by the workers concerned.

When calcium is present in abnormally large amounts there is a tendency for iron, zinc and boron not to be utilized efficiently by the plant even if present in normal or abnormally large amounts. Some other tests are therefore necessary. Large amounts of phosphorus and probably other elements have similar effects.

Plant injection is based on a few simple facts. When a plant is cut, far from bleeding, it usually absorbs any water or watery solution bathing the cut. Liquid absorbed in this way travels along paths that can be predicted. By making the cut in the correct position it is possible to permeate a selected portion of a plant and leave a similar neighbouring portion untreated for comparison. The effect of the injected substance is seen in the course of a week or so as an improvement in leaf colour or an increase in growth or both. Such improvements are usually, but not always, preludes to improvement in commercial performance.

As the methods have been described in detail elsewhere, only a general description of a few of these need be given here. For all of them actively growing leaves are desirable (and usually essential) for success. It is no easier to make a mature leaf respond to injection than to teach an old dog new tricks.

Interveinal injection. If a small wound be made equidistant between two side veins and the midrib of, say, an apple leaf and the wound kept moist by a suitable wick passing through it and immersed in a nutrient solution, the solution is sucked into the leaf and permeates an area bounded by the midrib and the two side veins for at least half their length. The effect of the solution can then be observed by comparing the permeated interveinal area with the one on either side of it. The treated and untreated areas are separated by a thin vein only. The slightest change in colour is therefore easily detected. Iron deficiency has been diagnosed in an almost completely yellow leaf in 2 days. If the leaf is an 'off colour' green a change in colour is usually not visible for 5 days or a week. An increase in growth is recognized by a puckering of the surface of the permeated interveinal area.

Leaf-tip injection. Cereals and other similar monocotyledons may conveniently be treated by splitting a suitable leaf in half lengthwise for half its length by pushing a pin through the mid-point of its midrib and running it along to the tip. The tip of one-half of the leaf is then immersed in liquid held in a suitable container and secured in position. The solution is sucked back for about half the length of the leaf and the effect may be watched by comparing the treated with the untreated half.

In a number of experiments on cereals a slight improvement has followed the injection of water. It is therefore necessary to do a water injection as a 'control' in such experiments, preferably on the second half of the same leaf.

Leaflet-stalk injection. If the blade of a leaflet, half way along the side of, say, a potato leaf, is cut off and the leaflet-stalk bent so as to dip into a suitable container of liquid, the liquid is sucked back into the leaf. If the container is removed after an hour, the leaflets immediately above and below the injection point are the only ones that are permeated. The effect of the absorbed liquid may be observed at the end of a week or fortnight by comparing these two permeated leaflets with the two corresponding ones immediately opposite them on the other side of the midrib.

Leaf-stalk injection. If the blade of an apple leaf be cut off and the leaf-stalk remaining on the shoot be bent so as to immerse the cut end in solution held in a suitable container the solution is sucked back into the shoot and is distributed according to a definite pattern in the leaves above and below. Usually the basal part of the first and second leaves above and below the injection point is permeated on the side nearer the injection point and is unaffected on the more distant side.

The apple, pear, plum and peach are all affected in this manner.

Branch injection. Single branches may be injected independently of the rest of the tree. In this way it is possible to observe the effect of a treatment on the fruit and on shoot growth as well as on the leaves. On a spur-pruned apple tree it is often possible to find twenty or more comparable branches for this purpose. The substance may be injected in either liquid

or solid form. For the liquid method a hole about one-eighth the diameter of the branch is bored diametrically through at a suitable point and connected by rubber tubing to a reservoir containing the liquid. Enough liquid is usually absorbed in about a day. For the solid method more than one hole is usually necessary and of larger diameter than for the liquid method. Into each hole is inserted the correct number of tablets of the substance and the hole is closed with a cork. The tablets and cork are conveniently inserted with a specially constructed instrument.

Whole tree injection. Similar methods to those just described may be applied to whole trees either experimentally or, in exceptional circumstances, on a commercial scale.

Applications of methods

A few examples of the results already obtained will indicate the future possibilities of these methods.

Boron deficiency in apples. The first really big achievement of these methods was the cure of perhaps the most serious trouble in apples in Canada, New Zealand and elsewhere. This was done by Atkinson (1935) in New Zealand; McLarty (1936) and Young & Bailey (1936) in Canada; and Jamalainen (1936) in Finland (see Roach, 1938). Each of these workers injected various solutions into branches or trees and found that each solution containing boron lessened or caused the disappearance of the 'corky' symptoms in the fruit. The following season soil applications were found effective except on highly calcareous soils, where spraying was effective. Thus in a short space of time a commercial cure was found for a trouble so serious as to seem to threaten the apple growing industry in parts of Western Canada. Here not only was a large part of the crop ruined by the 'corkiness' of the flesh of the fruit, especially near the core, but whole plantations were dying.

This 'corkiness' of the fruit is the only distinctive symptom of this disease in the apple tree. But lucerne, a cover crop commonly grown in Canada, shows characteristic symptoms which are used successfully for diagnosing the deficiency in the cover crop and indirectly in the apples growing above. This is, therefore, a convenient place for mentioning certain facts which indicate that great care must be exercised in the use of such 'indicator crops' for the diagnosis of mineral deficiencies.

Care necessary in use of 'indicator crops'. The assumption underlying the use of indicator crops is that different sorts of plants growing in the same soil will be affected by the same deficiencies.

A row of willow cuttings grew in alkaline peat soil with a row of potatoes 2 ft. away on one side and oats the same distance on the other. The potatoes were affected severely by a deficiency diagnosed by symptoms and by leaf injection as that of manganese. The oats were affected even more severely by the same deficiency which was diagnosed by the same methods and cured by spraying. The willow leaves were affected very severely by symptoms that were closely similar to those of manganese deficiency in other plants. Yet the results of leaf injection left no doubt that there was no deficiency of manganese in the willows but a severe deficiency of iron.

Copper deficiency in apple and pine trees in Western Australia. Workers in Western Australia have proved by injection methods that serious and extensive dieback in apple and pine trees there was due to copper deficiency. Soil applications proved effective and the trouble has ceased to be serious commercially.

Manganese deficiency in cherries. Growers in the Sittingbourne district were seriously troubled by the fact that many trees, just when they should have come into full bearing, ceased cropping and a little later began to die back. One large grower over a period of some 30 years had consulted all the experts who, he thought, might be able to help and the trouble had been ascribed to various causes, including faulty drainage which commonly accompanies it. This grower alone had grubbed some hundreds of trees.

Spectrographic analysis revealed that manganese was lower in the leaves of affected trees than in those from neighbouring productive ones. The cherry has been a difficult subject for diagnostic injection because it does not always absorb liquid when injected in the usual manner. The results from this method were not clear cut but they suggested that there was a deficiency of both manganese and iron.

Separate branches were therefore injected in the winter by the solid method with manganese sulphate, ferrous sulphate and manganese sulphate plus ferrous sulphate respectively. The manganese sulphate produced a most spectacular improvement in the foliage that developed in the summer and a heavy crop of cherries was produced. Whole trees were next injected. Many of these that had borne no crop for years then bore twenty or more 'halves' of cherries. As a result of co-operation between advisory and research officers, many hundreds of cherry trees were injected by the growers to get a quick cure. In the meantime, spraying had been proved to be effective. Spraying must be repeated every season and injection every four years. Growers seem likely to prefer spraying. Injection, especially by the solid method, is not to be recommended if any other method is effective because it is rather laborious and entails mechanical damage to the main stem of the tree. But until alternative methods were available growers were only too glad to bring their trees back into cropping by the solid injection method.

Other methods were also tried. These included the application of manganese sulphate to the soil. These all proved either ineffective or less effective than injection or spraying.

As a result of curing trees by injection and observing the leaf symptoms, it is now possible to diagnose manganese deficiency in cherries by such symptoms. A slight qualification of this statement, however, will be made later.

The examples so far given have been relatively simple in that they each were concerned with the deficiency of a single element. The remaining examples will show deficiencies of more than one element at a time.

Calcium-magnesium deficiency in potatoes. Early in the war, the Devon War Agricultural Committee ploughed up well over 10,000 acres of heath and other derelict lands. Over many hundreds of acres potatoes were a complete failure. The team was asked to help. Most of the worst potato plants were badly stunted but exhibited no symptoms characteristic of any deficiency. Leaf samples from the poor areas all showed a low calcium content and rather more than half a slightly low magnesium content also. The preliminary tentative diagnosis was, therefore, calcium deficiency and possibly, in places, magnesium deficiency. The W.A.C. Officers responsible for the potatoes quoted the dictum 'Potatoes do not need lime'. Leaflet-stalk injections were done at the same time as the leaf samples were taken. On all the poor areas there were strong responses to calcium chloride and on those with a low magnesium content to magnesium sulphate also. The W.A.C. Officers were impressed by the responses but said that it did not necessarily follow that an improvement in foliage growth and colour would lead to an increase in crop. Their attitude to both diagnoses seemed quite reasonable and proper, and as soon as possible trial diggings were carried out to determine how far yield was correlated with calcium content. The yields were quite according to prediction. The W.A.C. then acted with extraordinary energy. They applied limestone dust to the huge area for which it was advised, and an extra 3000 tons of potatoes were gathered the next season in consequence. Curing the calcium deficiency was estimated to have added six tons per acre of ware potatoes and curing the magnesium deficiency an extra ton per acre. These results followed a most happy co-operation between the W.A.C. Officers and the research team.

Perhaps the most striking fact revealed by this piece of work is that a mineral deficiency, so severe as to cause a complete failure of crop, may not be accompanied by any distinctive symptoms. As already stated, the plants were stunted but in other respects appeared quite normal.

An apparent multiple trace element deficiency in cherries. Not far from the manganese

deficient cherries already described, there are growing cherries exhibiting leaf and other symptoms that are similar to, but not quite identical with, those already mentioned. The trees are very variable and only a few whole tree injections were done. The results were as follows:

Manganese	Striking improvement in the foliage colour
Iron	Definite improvement but less marked than that due to manganese
Manganese + iron	Better than either alone
Zinc	Better than iron but less good than manganese
Manganese + iron + zinc + boron	The best result of all. Leaves apparently perfectly healthy

These results suggest at least a triple deficiency but they are insufficient to establish the degree of practical importance of the different deficiencies. Further curative work will be necessary to test this.

Manganese and other deficiencies in peaches in South Africa. The foliage of many hundreds of peach trees in South Africa in 1938 was completely yellow and the trees had ceased bearing. Many hundreds had already been grubbed. On one estate the results of leaf-stalk injections suggested that manganese deficiency was the main cause of the trouble and iron deficiency a contributory cause. A few whole trees were injected with solutions containing both manganese and iron to determine the correct dosage. The grower concerned then organized the injection of over 300 peach trees by 'Cape coloured' labour at an inclusive cost of 3*d.* per tree. The following season these trees bore a full crop of peaches.

A deficiency of zinc was already being cured in these plantations and elsewhere in South Africa by routine spraying.

Extensive leaf analysis and leaf injection experiments carried out during a year's stay in the country revealed a number of deficiencies, the most widespread of which were manganese, zinc and iron. These commonly affected the same trees, sometimes one deficiency, say manganese, dominating one part of a plantation and zinc another part of the same plantation.

Potassium plus trace element deficiency in fruit trees. As a result of war-time restrictions on the distribution of potassic manures, large areas of fruit trees are so deficient of potassium that they are likely to die before manure applied to the soil can become effective, unless some interim action is taken to save them. In the circumstances solid injection seems worth trying as an emergency measure.

In addition to the very striking leaf symptoms of potassium deficiency, there are commonly much less obvious ones of either manganese or iron deficiency. Leaf analysis has confirmed these trace element, as well as the potassium, deficiencies, and has also revealed apparent trace element deficiencies in many cases where only potassium deficiency symptoms were observed.

Results so far obtained suggest that it is even more important to cure the trace element, than to cure the potassium, deficiency. Curing the trace element deficiency seems to lead to a partial cure of the potassium deficiency without the addition of any potash. The best results have been obtained from treatment with trace element and potash.

This also is collaborative work between advisory and research officers. Much of it is being done in Essex.

Multiple trace element deficiency in arable crops. Early in the war large acreages of marsh pasture were ploughed in South Kent. In nearly every field the arable crops varied most markedly in growth within short distances. Land raised only a few inches above the general level bore heavy crops whereas land in the hollows, even if only a few inches below the rest, bore poor crops or no crops at all. Workers at Wye had observed marsh spot in peas in the neighbouring Romney Marsh and had proved this to be due to manganese deficiency. Symptoms of manganese deficiency were noted in wheat, oats, barley, peas, potatoes and mangolds on the freshly ploughed areas. The symptoms usually increased in severity as the yield decreased. A number of experiments were therefore carried out to test how far curing

this deficiency would raise the cropping to a general high level. The following are a few typical results:

TABLE 1. *Effect of spraying with manganese sulphate solution on yield of various crops and their increase in value*

	Yield per acre		Increase in		
	Unsprayed	Sprayed	Yield	Value	Cost
	cwt.	cwt.	cwt.	£	£
Wheat	18·5	35·5	17	12	3
Barley	22	36·5	14·5	18	2
Peas	9	20	11	26	1
	tons	tons	tons		
Parsnips	16·5	21	4·5	80	1·5

It will be seen that, even on this potentially fertile soil, the increases are large in comparison with the yields of the untreated crops and that the financial value of these increases exceeds the cost of the treatment many (from 4 to 50) times over.

Mineral deficiency in plants showing no symptoms. An even more striking result of the present work was the fact that increases of the order of 10 cwt. of wheat grain per acre were obtained by spraying crops showing no characteristic symptom of deficiency. The deficiency was diagnosed by plant analysis and plant injection and the effect of the spraying was statistically highly significant.

There was also a marked effect on crop quality as well as on yield.

TABLE 2. *Effect of spraying with manganese sulphate on yield and grain size of wheat*

Size	Unsprayed cwt./acre	Sprayed cwt./acre
Large	2·5	7·8
Medium	11·5	22·5
Small	4·5	5·2
Total	18·5	35·5

The detailed results, however, did not encourage the hope of raising the yield of the poorest areas to that of the best. Other deficiencies were therefore sought by plant injection methods.

Multiple trace element deficiencies. In addition to manganese deficiency, plant injection revealed in a number of crops deficiencies of iron, zinc, boron, copper and nickel, none of which was indicated by any symptom. As a result of complicated field experiments Mr C. Barclay and Miss H. M. Holmes obtained economically important increases in crop by spraying each of these elements on each of the three crops tried: viz. wheat, potatoes and broad beans. The sum of all these increases in crop was of the right order of size to raise the level of the poorest areas to that of the heaviest ever recorded. Only further detailed field work will determine how far this most desirable end can be attained.

Almost the whole supply of artificial manures is used for curing symptomless major element deficiencies which are almost universal. Evidence is accumulating that suggests that symptomless trace element deficiencies may be equally widespread and equally important.

Importance of complete diagnosis. The above results illustrate the importance of complete diagnosis. A particularly striking example was furnished by a field of potatoes. These showed marked symptoms characteristic of manganese deficiency; and there was a spectacular improvement in foliage colour following spraying with manganese sulphate, but little or no visible effect of spraying with zinc sulphate. Yet there was no yield increase resulting from the manganese spraying. There was, however, a slight increase in yield (about 1 ton/acre) following the zinc spraying. But a combined manganese and zinc spray raised the yield

from 7 to 11 tons/acre. Thus spraying according to diagnosis from the severe leaf symptoms was fruitless. The detailed results of the experiments suggest that spraying with the correct mixture of all six deficient trace elements might have doubled the crop.

Curious results with mangolds. The most severe leaf symptoms of manganese or any other deficiency ever seen by the writer were on mangolds that bore the exceptionally heavy crop of 70 tons/acre of good quality roots. Leaf analysis gave no clear indication, while leaf injection gave a suggestion of manganese and other trace element deficiencies but not strong enough to form the basis of a definite preliminary diagnosis. Spraying with manganese sulphate led to no detectable increase in crop. Symptoms were, therefore, seriously misleading and this is the nearest approach to leaf injection leading to a misleading diagnosis.

In another experiment there was wide variability in both manganese deficiency symptoms and yield in the experimental area. Leaf injection gave a clear indication of manganese deficiency. The yields were less than half the 70 tons/acre just mentioned. The yield was highest where the symptoms were most severe. But the increase from spraying with manganese was highest on the areas where the symptoms were least pronounced.

Importance of measuring the effect of curative treatment on yield and quality

Many of the facts already given emphasize the paramount importance of testing the preliminary diagnoses whether based on leaf analysis, plant injection or other methods, by measuring in field experiments the effects of curative treatment on yield and quality of crop. There is at present no alternative to this procedure and there is little likelihood of any in the near future.

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TRACE ELEMENTS IN RELATION TO HEALTH OF LIVESTOCK

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Introduction

The purpose of this contribution is to provide a brief survey of the practical significance of trace elements in relation to the health of the larger domesticated animals, and experimental observations on small laboratory animals are therefore omitted. The literature on the subject

* Contain references to publications on the use of plant-injection methods by other workers.

is now extensive but, since numerous special reviews are available, it is unnecessary to offer individual citations. The recent general review *Minerals in Pasture, Deficiencies and Excesses in Relation to Animal Health*, published by the Imperial Bureau of Animal Nutrition, provides an extensive bibliography and, although open to question on certain points of interpretation of data, is a valuable compilation for those interested in a world survey of the position up to the year 1944. In the limited time at disposal it is proposed to focus attention mainly on the position in Great Britain.

Definition

Since the term 'trace element' is sometimes used as if it meant 'micro-nutrient' it should be emphasized that it properly refers to any element which occurs in minute amounts in living tissues, whether it is known to exercise any function or not. The plant, in absorbing essential major mineral nutrients from the soil, incidentally takes up numerous other elements merely because they are brought into solution by the action of its roots, and since plant food is the basis of animal life the same elements are found in animal tissues.

General

If such an element is found to be locally concentrated in some particular tissue as, for example, iodine in the thyroid gland, the tendency is to assume a function for it although it may be long before precise knowledge of that function is gained; but when the quantity of the element is not only exceedingly small but is distributed throughout the tissues with no known sites of predilection, the impulse is to treat its presence as fortuitous until some instance of apparent absence is encountered in the form of an obscure deficiency disease, such as the 'pining' of lambs caused by lack of cobalt, or until the effects of unusual excess are manifested in the form of a toxicosis such as the cattle disease on the 'teart' pastures in Somerset caused by excess of molybdenum.

Many of the trace elements found in living tissues are apparently inert. For example nickel, although always present in soil-grown vegetation, does not appear to be an essential micro-nutrient and relatively large amounts are harmless to animals. Some are important to plant physiology but of little consequence in animal economy. Boron is a limiting factor in plant health but, if essential for animal life at all, the smallest amounts ever found in plants are sufficient in the sense that no boron deficiency disease has ever been recorded in animals. Zinc is certainly required by both plants and animals, and in the latter it is the metallic component of the enzyme 'carbonic anhydrase' found in considerable concentration in the red blood corpuscles and other tissues, but the quantities found in plant food exceed animal requirements and no naturally occurring zinc deficiency disease has been encountered.

With other trace elements the position is reversed and their significance in animal physiology is much greater than in plant economy. Cobalt, if of any significance in plants at all, is required in such small amounts that a pasture may be growing luxuriantly and yet induce emaciation and death in grazing ruminants, while selenium can be tolerated by growing plants in amounts which are exceedingly harmful to animals consuming them.

In selecting particular trace elements for special discussion attention may be confined to those of proved economic importance. These are iodine, copper, cobalt, manganese, selenium molybdenum and fluorine. Lead and arsenic are omitted because neither biologically essential nor absorbed through plant roots in quantities injurious to animals. A few years ago foetal intoxication by lead, occurring naturally in pastures, was considered as a possible cause of congenital enzootic ataxia of lambs, and is still considered as a contributory factor by some workers, but its significance is very doubtful. As for arsenic, the danger of cumulative poisoning is much exaggerated. Substantial sub-toxic doses repeatedly administered to sheep are rapidly eliminated, and the amounts naturally present in oysters and flat fish, although well above the limit sanctioned by health authorities concerned with arsenic as a food contaminant, are unconsciously consumed without detriment to human health.

Iodine. It is hardly necessary to mention that iodine is required for the formation of thyroxin, the hormone of the thyroid gland, and that goitre is the common sign of dietary deficiency. Daily requirements, however, are small, of the order of 50–100 μg./100 lb. body weight, and iodine deficiency in acute form is rare except in certain well-defined areas of the world. One of the best known earlier cases of acute deficiency occurring on a large scale was recorded from certain American states in 1917, in which 'foetal athyrosis' or 'hairless pig malady' occasioned heavy losses until the cause was identified and the trouble dissipated by including traces of iodine in the rations of the breeding sows. Piglings were still-born, or born hairless, and died shortly after birth even when the mothers were apparently healthy. Congenital thyroid hyperplasia in lambs is common in iodine-deficient areas, but goitre also occurs in older animals and has been reported in cattle, sheep, goats, horses, and pigs. Incidence in the U.S.A. has been mapped and iodine deficiency reported from no fewer than 14 states. Goitre in livestock has also been recorded in S. America, Canada, New Zealand, Finland, Switzerland, Norway, Sweden and Germany. Occurrence in Great Britain is sporadic and although the problem of iodine deficiency is not important in British farm practice the question of 'iodine intake below the optimum' merits further investigation.

In general the degree of enlargement of the thyroid is inversely related to its iodine content, once a certain 'critical level' has been reached. For lambs this is about 0·03 % on the fresh gland. Below this the gland increases in size as the iodine content diminishes, sometimes reaching a weight 50 times the normal, with percentage iodine values down in the fourth decimal place.

It should be mentioned, however, that direct iodine deficiency is not the only cause of thyroid hyperplasia and any factor which reduces the absorption of iodine or increases the utilization of thyroid hormone may also cause goitre. Continuous administration of certain organic cyanides may be goitrogenic by depressing tissue oxidation and so producing a compensatory reaction on the part of the gland, and goitrogenic substances may be present in plants. A 'goitrogenic factor' has been reported in cabbage.

In practice, thyroid hyperplasia is easily controlled by the use of iodized salt, care being taken to avoid undue excess which may lead to 'iodism'. Supply of iodine in inorganic form in amounts above those required by the normal thyroid gland is of no value, although thyroxine itself, or 'iodinated proteins' possessing thyroxine activity, can enhance milk production of dairy cows by artificially elevating metabolism at particular periods of lactation.

Copper. Diseases associated with low copper in pastures have now been reported in many parts of the world. Although this deficiency was first recorded in Holland in 1933 the most valuable pioneer work has been carried out in Australia, where 'coast disease' of ruminants, chiefly sheep, is caused by dual deficiency of cobalt and copper. The co-called 'falling disease' of cattle in south-western Australia, characterized by cachexia, anaemia, suppression of oestrus in cows, and stunted abnormal growth of calves, is regarded as a simple copper deficiency. Affected pastures show a copper content as low as 1 p.p.m. in the dry matter of the grass, as compared with 5–15 p.p.m. for healthy pastures on which sick cattle recover when transferred. Blood copper is low, down even to one-tenth the normal value. Sheep are also affected and 'stringy wool', a condition in which the fleece loses bulkiness and becomes straight, limp and glassy, is common in copper-deficient areas.

Of more insidious character is the 'enzootic ataxia' of lambs, a demyelinating disease affecting primarily the cerebrum of the foetus carried by outwardly healthy ewes, and preventable by administration of small amounts of copper sulphate during pregnancy.

A so-called 'licking disease' of ruminants, attributed to copper deficiency, has been described in Schleswig-Holstein, similar to that occurring on the sand and moor soils of the Eastern Provinces of the Netherlands.

Copper deficiency in cattle has not been reported in Great Britain in the characteristic form of 'falling disease' or 'liksucht', but a hypocupraemic condition occurs sporadically, in which copper values in the blood are almost down to the levels recorded in Australia.

Two widely separated farms in England are at present under investigation in which blood values of grazing cattle are only 0·02–0·04 mg.% in place of normal values of about 0·12 mg.%.

The disease 'swayback' in lambs is, however, widespread in England, Scotland and Wales, and corresponds pathologically to the 'enzootic ataxia' of Australia. On certain farms in Derbyshire the incidence varies from 10 to 30% of the total lamb crop in different years but may even reach 90%. The symptoms are usually evident at birth and are essentially those of spastic paralysis with resultant inco-ordination of movement. The characteristic pathological change is a diffuse symmetrical demyelination of the cerebrum and acute cases show gross cavitation of the whole hemispheres. Secondary degeneration of motor tracts in the cord is always present. The disease is of pre-natal origin and acute cases rarely survive the first few days of life. If the lesions are slight and the lamb is able to keep up with its mother it may survive, improve, and reach adult life still showing inco-ordination of movement. In areas where incidence is low some of the clinical cases may be of a 'delayed type' in which inco-ordination of movement is manifested several weeks after birth.

Although the ewes themselves are apparently healthy and anaemia is rarely shown, blood copper values are always low, most commonly below one-third normal. It does not follow, however, that an individual ewe with very low blood copper, down even to one-tenth of normal, will deliver a swayback lamb and sometimes one twin lamb may show the disease and the other not. All that can be said is that if the blood copper status of a flock of ewes is low, the incidence of swayback in the lambs is likely to be high, and that if this low status is raised by administration of copper salts during the later stages of pregnancy the disease will disappear. Prevention of swayback in the lambs may be accomplished by oral dosage of the ewes with copper sulphate at intervals of a few weeks from mid-gestation onwards, or by exposure of copperized salt licks or mineral mixtures in hooded troughs and training the sheep to consume them. Although not a practical measure of control, demyelination in the foetus can be prevented by a single injection of 20 mg. of copper (as the sulphate) into the jugular vein of the mother 8 weeks before lambing. Sufficient copper is stored in the tissues to tide over the intervening period.

The most interesting feature of English 'swayback' in comparison with Australian 'enzootic ataxia', is that it is not necessarily a simple copper deficiency and that some other unknown factor enters into the etiology of the disease. Although in Australia the copper content of affected pastures is below 5 p.p.m. the apparently identical disease in England occurs on pastures of normal copper content. From one farm in Derbyshire showing an average of 15 p.p.m. in pasture dry matter, twenty ewes, each with low blood copper and a previous history of swayback, were transferred to the Veterinary Laboratory at Weybridge and fed in stalls on a copper low ration supplying only 5 p.p.m. The blood copper rose rapidly and all lambs were normal. In the control group left on the farm in Derbyshire, with the higher daily intake of copper, blood copper remained low and incidence of swayback was 20%. The following year twenty similar ewes were transferred from the same farm along with farm hay to serve as roughage in the Weybridge ration. This time the blood copper remained low and three cases of swayback were obtained, an incidence of 15%, similar to that amongst the controls on the Derbyshire farm in the same year.

Such observations indicate that there is some other factor in the vegetation of affected farms which either reduces the availability to the animal of the total copper present, or interferes with the copper metabolism in the animal itself. The nature of this factor, organic or inorganic, is still unknown. High lead in pastures has been suggested as conducive to swayback but the available evidence is against that view. Deliberate administration of lead during pregnancy to ewes on normal diets did not reduce the level of blood copper.

Cobalt. The classical work on cobalt deficiency comes from Australasia where, about 12 years ago, 'enzootic marasmus', a wasting disease of sheep and cattle in Australia, similar to the 'bush sickness' of New Zealand formerly attributed to iron deficiency, was found to

be due to lack of cobalt, and rectified by the traces of that element in the crude iron oxide used for prevention and cure. Daily doses of as little as 0·1 mg. of cobalt for sheep, or 1 mg. for cattle, in the form of a pure soluble salt, were found to maintain animals in good health on affected farms. 'Coast disease' was subsequently found to be caused by dual deficiency of cobalt and copper, a feature also characteristic of the 'salt sick' of Florida. Since then similar diseases have been reported from several parts of the world, a peculiar feature being that the adverse effect of cobalt deficiency seems to be confined to ruminants and is not shared by horses if these happen to be reared on the same pastures. The critical level for cobalt in pasture dry matter is given by New Zealand workers as about 0·05 p.p.m., with a margin of about 40% either way, i.e. pastures containing more than 0·07 p.p.m. are safe and those containing less than 0·03 p.p.m. always dangerous. Top dressing of pastures with soluble cobalt incorporated in fertilizers was found effective in areas where the system of grazing rendered this practicable. In other areas reliance is placed on mineral licks containing cobalt, or on systematic oral administration at suitable intervals.

In Great Britain 'enzootic marasmus' of cattle does not appear to have been specifically recorded except on the island of Tiree, but 'pine' or 'vinquish' of lambs in Scotland, attributed to iron deficiency in 1928, was shown to be due to lack of cobalt in 1937 when Australasian observations became well known. Since that date the most valuable work has been done by collaboration between the Moredun Institute of the Animal Diseases Research Association at Edinburgh and the Macaulay Institute for Soil Research at Aberdeen. Large tracts of land north of Inverness are affected and specific districts in the Solway area and Roxburghshire in the South have been defined. A map of the known 'cobalt pining' areas in Scotland is given in a paper by Stewart *et al.* in the July 1946 issue of *The Empire Journal of Experimental Agriculture*. In England the areas most definitely affected are the granitic soils of Devon and Cornwall recently investigated by Patterson at Dartington Hall.

Symptoms of 'cobalt pine' are those of progressive debility. In lambs growth is markedly retarded, the fleece becomes lustreless and broken, there may be serous discharge from the eyes, physical condition deteriorates until finally, as a result of extreme weakness, the animal is unable to rise. Mortality rate may reach 30% on some farms, with serious loss of condition in most of the flock. Suitable change of pasture results in rapid recovery, and was the method of control practised before the etiology was understood and cobalt therapy adopted.

In areas in which 'pine' is less acute and actual mortality negligible, economic losses may still be very great and lambs which appear to grow normally during the first 2 months of life may fail to make headway thereafter and at the autumn sales weigh less than two-thirds of similar lambs receiving a cobalt supplement equivalent to about 1 mg./day, either administered orally at weekly or fortnightly intervals, or supplied as component of a mineral mixture in hooded troughs, or provided indirectly by dressing the pastures with cobalt chloride at the rate of about 2 lb./acre.

The Scottish workers place the safe figure for cobalt content of pasture dry matter at about 0·08 p.p.m. and consider it highly unlikely that healthy lambs can be reared on pastures showing less than 0·05 p.p.m.

Since it is often difficult to secure pasture samples free from a degree of soil contamination which may seriously invalidate analytical results, the Macaulay Institute prefers soil analyses for survey purposes. Figures for total soil cobalt have little significance but the moiety extractable with 2·5% acetic acid is regarded as a fairly reliable index. The range 0·25–0·30 p.p.m. for acetic-soluble soil cobalt is treated as critical.

Manganese. From experimental work on small animals, manganese is accepted as an essential micro-nutrient but there is more than enough in the vegetative parts of plants and no manganese-deficiency disease has been definitely recognized in the larger domesticated animals. Certain forms of delayed ovulations in cows, where the female comes into heat and accepts the male before descent of the ovum, have been attributed to manganese deficiency in pastures occasioned by excessive liming of the soil, but the evidence is very doubtful.

Adverse influences of excessive manganese in pastures have also been suggested, for example, in 'forage anaemia' of horses in Scandinavia, but again the evidence is unsatisfactory.

Deficiency in grain-fed animals has been suspected with better reason and 'slipped tendon' or 'perosis' of chicks has been attributed to lack of manganese, has been produced experimentally with diets poor in manganese, and prevented by a manganese supplement, but other factors are also involved in the etiology of the disorder.

Selenium. The inessential element selenium is biologically important only because it is vastly more toxic for animals than for plants, so that on seleniferous soils healthy plants can accumulate quantities high enough to produce disease in animals consuming them. Plants capable of accumulating large amounts of selenium are said to act as 'selenium converters' in the sense that they readily absorb the element from seleniferous shales and return it in due course to the soil in more soluble form for absorption by other plants.

Selenosis has not been encountered in Britain, but in a few parts of the world, notably in South Dakota west of the river Missouri, it has occasioned serious disorders in all livestock including poultry. The so-called 'alkali disease' of this area manifested by depressed growth, loss of hair conspicuous in the mane and tail of horses, abnormal development and sloughing off of hoofs is now known to be due to selenium poisoning. Amongst poultry a conspicuous feature is non-viability of eggs or hatching of monsters. The safe limit for selenium content of grain is about 3 p.p.m. and the effect on the hatchability of eggs, of a content of 10 p.p.m. in the ration of fowls, is apparent from the seventh day onwards.

The 'blind staggers' of Wyoming is a still more acute form of selenosis, in which liver lesions lead to death before loss of hair and hoofs occurs.

Although the element is widely distributed in nature it is readily excreted in bile and urine, so that no real danger exists until a level of chronic intoxication is reached sufficiently high to produce irreversible damage to certain tissues. The urine of men employed in the extraction of selenium can contain a few p.p.m. without clinical symptoms appearing.

In known seleniferous areas measures of control are now adopted to safeguard the health of stock and prevent abandonment of farms which have acquired a bad reputation. These include eradication of plants which are 'selenium converters', replanting with forages which do not accumulate the element, improving soil drainage, and obviating overstocking to permit of selective grazing.

Molybdenum. The element molybdenum is widely distributed in traces in pastures, and although important for the fixation of atmospheric nitrogen by the free-living *Azotobacter* in soils and the nodule bacteria of legumes, it has never been regarded as essential in animal life. Like selenium, however, it can be taken up by healthy plants in quantities injurious to animals. The clearest case of molybdenosis of farm stock is the so-called 'teart' pastures on the molybdeniferous lower lias geological formation in Somerset. These 'teart' pastures cause a scouring disease affecting ruminants, particularly cows in milk and young stock. Scouring may start within 24 hr. of putting the cattle on to affected pastures. The dung becomes extremely loose, yellow-green in colour and evil smelling. The animals soon become filthy, develop staring discoloured coats and lose condition rapidly, eventually suffering permanent injury or death. The rapidity with which scouring stops and recovery ensues on transfer to non-teart pasture is very characteristic. Sheep are not so seriously affected although they become loose and dirty. Horses are not affected at all.

The area concerned in central Somerset comprises about 20,000 acres and smaller areas are found in north Somerset, Gloucester and Warwick. 'Teartness' has been known for over a century but it was only in 1938 that the cause was found by spectrographic examination of affected pastures at Jealott's Hill Research Station. The finding of high molybdenum was promptly checked by feeding sodium molybdate to cattle and reproducing the disease; also by dressing healthy land with molybdate and reproducing teartness in the pasture.

The Jealott's Hill workers found that non-teart pastures rarely exceeded 5 p.p.m. molybdenum on a dry-matter basis, whereas in teart pastures values up to 100 p.p.m. were

obtained. The severity of scouring was directly related to the content of water-soluble molybdenum in the grass. A daily dose of 2 g. of copper sulphate per beast was found to be an effective antidote and use of copper sulphate enables cattle to graze on teart pastures with impunity for the whole of the customary grazing periods. The mode of action of the copper sulphate is not clear but it is not related to any copper deficiency. Copper content of pastures is normal and blood copper of scouring cattle is not diminished. The disease can be reproduced at will within a few days and as rapidly cured by removing the cause. The mode of action of the molybdenum is not yet understood but the rumen appears to be at least the initial centre of the disturbance. Non-ruminants are not affected and rabbits can stand very large doses of sodium molybdate without change in the consistency of the faeces.

A few years ago a form of bovine haematuria in South Australia was credited to long continued ingestion of molybdenum in quantities much smaller than those encountered in Somerset but the theory has since been discredited. Recent work in Australia suggests that long continued ingestion of molybdenum may reduce copper storage in the liver but the significance of the observations is in doubt.

Fluorine. Although fluorine occurs in appreciable quantities in bones and teeth it is not regarded as an essential micro-nutrient, and until recently it was considered entirely harmful and only unavoidably present in animal tissues. Traces are now regarded as useful in protecting against dental caries and water supplies containing up to 0·5 p.p.m. are regarded as beneficial although levels exceeding 1–2 p.p.m. are definitely harmful. Its significance in animal health only arises when larger amounts are ingested and this usually occurs by contamination rather than by the presence of abnormally large amounts of fluorine in vegetation, the content of which remains low even when grown on soils very high in the element.

Spontaneous fluorosis is found in many parts of the world, the most notable diseases being the 'darmous' of the rock phosphate areas of Morocco and Tunis and the 'gaddur' of the volcanic soils of Iceland. Darmous is particularly common in sheep, which may die of inanition caused by wearing down of the incisors and dystrophy of all the permanent teeth. The use of ground rock phosphate as mineral supplement for cows has occasioned severe fluorine cachexia accompanied by reduced milk yield, lameness, exostoses on long bones and mandibles, hypoplasia of dental enamel and other changes.

The most serious outbreaks of fluorosis in Britain have been of industrial origin and caused by contamination of pastures for a mile or so in the direction of the prevailing winds from large factories emitting fluorine compounds. One such outbreak has been reported from the vicinity of aluminium works near Fort William in Scotland, the source of fluorine being the cryolite used as flux. Another is the outbreak in Bedfordshire studied in 1939 in the vicinity of a large concentration of brick kilns. Yet another has recently been studied in the vicinity of open-air burning of ironstone in Leicestershire. In both the latter cases pasture contamination was very serious in the immediate vicinity of the source and affected cattle showed fluorine values in the urine over 10 times the normal, and deposition of fluorine in bones up to 40 times the normal figure, with consequent lameness and cachexia.

REVIEWS

The New Genetics in the Soviet Union. By P. S. HUDSON and R. H. RICHENS. Pp. 88. Cambridge: Imperial Bureau of Plant Breeding and Genetics. 1946. 6*s.* 0*d.*

The history of genetics and its development varies widely from country to country. In the United States of America it has been a story of quick appreciation leading to steady expansion of both teaching and research facilities. In Great Britain development has been much slower. Though playing a predominant part in the early development of the science, this country has to-day only a small number of geneticists: teaching is often in the hands of non-specialists, and research facilities are meagre.

In Russia there has been still another story to tell. Following the October Revolution, genetics, under the leadership of Vavilov, received great encouragement and progressed by leaps and bounds. The phyto-geographical work which resulted, and which gave to Vavilov world fame, needs no recounting. The situation began, however, to take a new turn in 1933. It was felt that genetics was not pulling its weight in improving varieties and raising the standard of seed production. By 1935, Lysenko, already well known for his physiological work on phasic development, began an all-out attack on genetics and in a series of controversial conferences, he and his followers gained the upper hand. Vavilov lost control of the chief genetical centres to Lysenko, and died, officially discredited, under obscure circumstances at a date not known with certainty but believed to be in 1942.

The course of these events has been known only in the barest outline to western scientists. The relevant publications have been in Russian and scattered through a wide range of journals, many of which would not normally be consulted as sources of scientific information. Consequently it has hitherto been impossible to bring the facts together in the way necessary for the formation of a balanced opinion. This situation has now changed. Drs Hudson and Richens have performed the invaluable service of comprehensively surveying the literature on the subject and presenting it in a way which for the first time enables us to form a picture of Lysenko's objections to standard genetical theory, of his new genetical theory with its underlying facts and arguments, and of the means by which he has swayed Russian opinion in this new direction. And what a picture it makes.

In their first two sections the authors paint in the background both historical and psychological. Historically, Lysenko's school has arisen from the somewhat violent admixture of Dialectical Materialism, Soviet Darwinism, and Mičurinism with genetics. The biographical information and the reviews of existing genetical facilities and publication practice in the U.S.S.R. are a feature of this section.

The account of the psychological aspect to which attention is next turned will be of especial interest. The Russian methods of controversy, of judging the merits or demerits of a case, as revealed here will make strange reading for western eyes. Logical reasoning is mixed up with arguments that can best be described as alogical. They are not illogical, for logic does not enter into them. Indeed logically valid conclusions may at times be reached, but they are reached by means other than those of logic. Arguments of this kind are the appeals to a recognized authority, such as Darwin or Mičurin, for justification of a view, and the condemnation of opposition on the grounds that it rests on Lamarckism, Capitalism or some other basis regarded as heretical because it is inconsistent with the chosen authorities. Questions of interpretation of authority also enter into the arguments which, as Hudson and Richens point out, come to bear a striking resemblance to medieval theological controversy.

Motives of opponents are often called into question; sometimes, as when Prezent accuses biologists of using learned terms to mask ignorance, in ways with which it is impossible to

feel wholly out of sympathy. Not all terminology is, however, so to be condemned out of hand. Genetics has been widely regarded as a target suited to this type of criticism, and here the difficulty appears to lie as often in the novelty which many genetical ideas present to the non-geneticist as in the obscuring nature of the terms used. Lysenko and Prezent themselves have been led, in order to express their views, to introduce new terms or to redefine old ones in ways which could with every justification be described as obscure. A certain inconsistency in their use of these terms does nothing to clarify the issues at stake.

This background is essential to the understanding of evidence which Lysenko adduces in favour of his views. The evidence itself is examined in detail by Hudson and Richens who point out that much of it must be regarded as inadmissible by western standards, owing to a crippling lack of experimental design and statistical analysis, extending even to the absence of controls. This is a consequence of the antipathy expressed by Lysenko and his school towards mathematical treatment, which they regard as incapable of proving or disproving biological laws. Where the genetical facts conflict, as they often do, with Lysenko's expectations derived from *a priori* considerations of phasic development, they are reconciled by the statement that these expectations can be realized only under certain ideal conditions of existence, conditions for whose detection no means are yet available other than the conformity of results and expectation.

With this evidence ruled out as uncritical by the elasticity of Lysenko's theories, there remains that which might be taken as favouring his views in a more positive way. Most of this has, however, been assimilated already into modern genetical theory. So it too fails to afford a critical test. This is true even of the data on the genetic interaction of stock and scion in grafting experiments on which Lysenko has based much criticism of genetical theory. Here Hudson and Richens regard Lysenko's evidence as suggestive though by no means final, and they advocate wider experimentation to settle the question. Some of the claims in this direction will be viewed with doubt by geneticists; but the possibility of such interaction has already been canvassed, notably by Darlington, from a purely genetical standpoint.

Recognizing the ambiguity of the factual evidence, Hudson and Richens next turn to an examination of the actual theories of Lysenko and Prezent. These rest essentially on regarding the organism as a series of developmental stages, each proceeding by assimilating nutrients to give rise to its successor. A full cycle of stages constitutes a generation. The term nutrients is used in a very wide sense to cover even such things as pollen grains, and assimilation is regarded as selective, the biologically advantageous nutrients being preferentially absorbed. In this way the course of development is guided partly by the existing properties of the system and partly by the environment in the form of available nutrients. Once assimilated, nutrients become themselves part of the system and so help to determine its future properties. Novel nutrients give new cycles of development, which then form part of the nature of the organism. Differences between nutrients locally within an individual lead to internal genetical differences and, if in the sporogenous tissues, to segregation. Hybridization, segregation, and inbreeding depression, are all interpreted in terms of these developmental potentialities, to which is added the notion of advantageous effects arising from the combination of dialectically opposed potentialities.

Now in the past it has been at once the strength and the weakness of genetics that attention has been focused preponderantly on the relations of whole organisms. The strength, because in this way the transmission and organization of the hereditary materials could be analysed unobscured by the main complexities of development and differentiation. The weakness, because these developmental processes, always assumed in genetics as the link between genotype and phenotype, were largely neither understood nor even examined in their genetical aspects. Genetical theory has therefore been grounded on the study of gene transmission. The problems of gene action have occupied a secondary place. Though their existence has always been appreciated, these problems have until recently proved intractable to geneticists by reason partly of technical limitations and partly of theoretical complexity.

Now, however, this situation is passing away. Growing attention is being paid to gene action in relation to development. Great advances, both experimental and theoretical have recently been made, and greater still may be confidently awaited.

It is to these causes that we must attribute the air of unreality which genetics appears to have worn in the eyes of many physiologists; and it is from these new lines of progress that the answer to their doubts will come. We may surmise that Lysenko has realized the past shortcomings of genetical theory in relation to development, while failing to realize that its mechanical foundation gave to genetics coherence and strength—a strength which may be limited in scope, but which is made none the less real by the assumption, rather than explanation, of the physiological relation between genotype and phenotype. He has therefore sought to develop a physiological theory of genetics in opposition to, rather than in extension of, the mechanical theory: to expand the part by replacing the whole. For this reason his theory is ineffectual. The complexities of hereditary transmission are not to be avoided so easily. He seeks, for example, to abolish the distinction of genotype and phenotype; but is continually forced to recognize it, though in a less helpful form, as a conservatism of matter in development, or as a difference between, in Hudson and Richens' translation, the 'soul' and the 'shirt' of the organism. The genetical understanding of development and differentiation will come more quickly and more certainly by building on the present foundation, in the way that Beadle, Caspersson and many others are now doing.

Lysenko's objections to standard genetical theory are even less impressive. That their theories should be regarded as heretical in either derivation or consequences will cause western geneticists little concern. That genetics has not yet fulfilled all its early promise of wide practical usefulness is realized by many geneticists, who are endeavouring to extend its applicability. Indeed it appears that this objection can be turned against Lysenko's own views, for there is little evidence that the results he forecast from their application—new varieties in three or four years—have ever been realized in practice. Many of Lysenko's other objections, while valid against the genetics of thirty years ago, fail against the genetics of to-day of whose developments Lysenko and his followers seem to be unaware. The objection which is shared in part by Hudson and Richens, that dauer-modification affords evidence against the distinction between phenotype and genotype, is to be answered similarly. The distinction is valid for the all-important nuclear inheritance. It begins to break down, as geneticists themselves would expect, in cytoplasmic inheritance. The criticism that the gene theory is an elastic hypothesis (a view also expressed particularly about polygenic theory by 'Espinasse in this country) lacks sting, because supplementary evidence of the kind required to establish its validity is in fact already available in the literature. And so we go on. Lysenko's objection to genetics is founded less on fact than on a false feeling that a physiological view must be antagonistic to existing mechanical theory. Its wide propagation in Russia has depended solely on skill in controversy and success in appealing to the authorities and to collective farmers.

As Hudson and Richens stress, the need is for accommodation between the physiological and the mechanical. This is coming from geneticists themselves, and we feel that Lysenko is not likely to speed it on its way. Thanks to Drs Hudson and Richens we can now base this conclusion on a fuller evaluation of Lysenko's statements. That the work of preparing their bulletin has been prodigious is shown by the list of references, particularly those in Russian now made available to us for the first time. They are careful to print the original text wherever matters of translation could be misleading. Though critical, they are never unfriendly to Lysenko and his followers. Indeed geneticists may feel that sometimes they err the other way, for their presentation of the genetical case is not always without blemish. This is, however, a minor objection. Our feeling must be one of gratitude to them for presenting a fascinating story in a way permitting us all to form our own opinions. Their work deserves a wide public, and one not confined to the professional geneticists.

KENNETH MATHER

Trace Elements in Plants and Animals. By WALTER STILES. Pp. xi + 189. Cambridge University Press. 1946. 12*s.* 6*d.*

During the second half of the nineteenth century the use by botanists of the water-culture methods developed by Sachs and Knop showed that sulphur, potassium, phosphorus, calcium, magnesium, iron and possibly sodium and chlorine were necessary mineral elements for healthy plant growth. At the turn of the century manganese was added and, by the early 1920's, zinc, aluminium, boron and silicon. More recently evidence has accumulated that copper and molybdenum may also be essential and, within the last decade, claims have been put forward for numerous rarer elements, many if not most of which are, perhaps, in need of confirmation. With animals the study has not advanced so far but it is now known that calcium, phosphorus, sodium, magnesium, potassium, chlorine, sulphur, iron, copper, manganese, zinc, iodine and cobalt are essential to animal life and, as with plants, claims have been made for many rarer elements.

The mineral elements required by plants and animals fall into two rather ill-defined groups: those which are regarded as nutritive elements since they are usually required in some quantity and take part in structural building and essential metabolism; and others called trace elements or micro-nutrients of which infinitesimal amounts usually suffice and which seem to function primarily as catalysts. With both plants and animals an excess, deficiency or imbalance of mineral elements may give rise to ill health which may end in premature death.

In this book Prof. Stiles confines his attention to the trace elements. Chapter I is a brief historical introduction to the subject. It concludes with a useful list, running to seven and a half pages, of micro-nutrients under each of which are given the species for which it has been claimed that the element either is essential or induces an increase in rate of growth or yield. To each plant is appended the name of the first worker to call definite attention to the favourable effect of the element. Chapter II on the investigation of micro-nutrient problems contains a critical account of the methods of purification of materials used in culture experiments and the estimation of the elements in plant material and ends with a brief discussion of the diagnosis of mineral deficiencies of plants. Chapter III is a straightforward account of the diseases of plants caused by deficiency of manganese, zinc, boron, copper and molybdenum and it might, with advantage, have been extended to include certain other elements. Chapter IV is an interesting discussion of the obscure problem of the functions of trace elements in plants. In these chapters Prof. Stiles is writing of work in his own field of research and he gives a good account of the present state of the subject.

The author is much less happy when dealing with trace elements in animals to which Chapter V is devoted. Fortunately, this subject has been admirably discussed by F. C. Russell in his bulletin *Minerals in Pasture: Deficiencies and Excesses in Relation to Animal Health* (Imperial Bureau of Animal Nutrition, May 1944). The preface to the book by Prof. Stiles is dated 28 October 1944 and it is regrettable that Mr Russell's bulletin was issued probably just too late for inclusion: this was probably also the case with Hoagland's *Inorganic Nutrition of Plants*. The book concludes with Chapter VI, an all too brief chapter in which the author summarizes the progress made and indicates promising lines of research.

The book makes no pretence of being other than a critical digest of the literature although the value of this is enhanced by shrewd comments based on the author's own experience. It is illustrated by seven Plates showing deficiency diseases of plants; there is a bibliography of 432 citations and a good index. A few statements requiring emendation occur in the text and the two values given at the end of the first paragraph on p. 5 need to be transposed. One is also a little surprised to find that the bibliography cites only the first edition (1931) of Miller's *Plant Physiology* whereas the second edition (1938) gives an account which in some ways is more detailed than that in the present book of the elements absorbed by plants.

The book by Prof. Stiles and the bulletin by Mr Russell taken together form an excellent introduction to this important and rapidly advancing subject. WILLIAM B. BRIERLEY

Actions of Radiations on Living Cells. By D. E. Lea. Pp. xii + 402. Cambridge University Press. 1946. 21*s.* 0*d.*

In 1936 there was published a treatise entitled *Biological Effects of Radiation*, comprising two massive volumes edited by Prof. B. M. Duggar, in which a galaxy of American specialists made a critical survey of this important field of inquiry (see *Ann. appl. Biol.* **24**, 216). In the ensuing decade enormous advances have occurred in our knowledge of this subject but especially, perhaps, in the quantitative study of the action of X-rays and other ionizing radiations on bacteria, viruses and the chromosomes and genes of animals and plants. It is quite impossible for any single person to cover the entire field and, in the present book, the author confines himself to a rather detailed consideration of the mechanisms of those actions of radiation which are sufficiently well understood for such a discussion to be now profitable.

In the first two chapters the relevant physical properties and chemical effects of ionizing radiations are described leading, in Chapter III, to the acceptance by the author of the so-called 'target theory'. In Chapters IV–VIII an excellent account is given of our knowledge of the inactivation of viruses by radiation, the genetical effects of radiation, the production by radiation of structural changes in chromosomes, and the mechanism of induction of such changes and of delayed division. The material is presented in some detail and is interpreted throughout in terms of the target theory. In these chapters the author makes out a good case for his opinion that 'the validity of the target theory is as certain as any scientific theory ever is in a rapidly developing subject, in the inactivation of small viruses by radiation, and the production of certain chromosome aberrations in higher cells' and, further, that its validity is 'highly probable' in the 'killing of large viruses and bacteria, and the production of gene mutations'. Chapter IX is devoted to the killing of single-celled organisms and of individual cells of multicellular organisms, and the author's attention is directed mainly to such lethal actions as may be understood in terms of the mechanisms he has described in earlier chapters.

As the study of the action of radiations on viruses, bacteria, genes and chromosomes is mainly quantitative the interpretation of the data involves considerable mathematics. The author has, however, as far as possible, kept the algebraic detail in the background and has provided ample graphs and tables which summarize a great amount of numerical data and which enable the experimentalist to interpret his own results along the lines suggested without any special technical mathematical ability being demanded. An appendix comprises a number of calculations, the results of which have been required in the text, but the details of which are of only technical interest. The volume closes with a useful bibliography of 483 citations, including researches published in 1945, and author and subject indexes. It is illustrated by four plates, 61 text-figures and 83 tables.

The book is an admirable survey of a particular field of investigation, namely, the application of radiation technique and the special point of view involved to a wide range of organisms and organic structures. The way in which the results of these investigations fall into line and point directly to the target theory is remarkable and is very well brought out by the author's clear exposition. There are, of course, as the author points out, many actions of radiation on living organisms which are not to be interpreted on the target theory, there are numerous controversial issues and there are many data which the theory fits very awkwardly but, both in general and in detail, the author's case for the acceptance of the theory is impressive. If it be true, then many of its implications are of almost revolutionary importance over wide ranges of biology.

Dr Lea has taken an active part in the recent exploration of this obscure field and his book is not only illuminated by his own fruitful experience but it contains a good deal of unpublished work by himself and his colleagues. The book is, however, not merely an exposition of work done in a particular field but is valuably suggestive of further research and application. The author has very definite views on many problems to which he does not

hesitate to give straight and forcible expression. Further, I find it a very welcome thing to meet in a book of this high calibre and obvious mastery of the subject, sentences beginning with such words as 'It is a plausible conjecture to...', 'A first speculation would be that...', 'We shall continue the discussion on the assumption that...', 'If one supposes...', 'It is probably true to say that...', 'This behaviour suggests that...', 'We imagine the...', 'If significance is attached to these analogies, one is led...', 'In this way it is possible to...', 'It is probably safe to neglect the...', 'If we are correct in arguing by analogy from...', 'If the view is correct that...', 'It is believed that...', and so on. It is an ingrained habit in most biologists to depreciate speculation, but I very often think that if biologists who are masters in their own field would rid themselves of this almost pathological inhibition and would speculate a little more freely, the biological sciences might undergo almost spectacular advances, advances as great as those to which speculation has led the physical sciences. Imaginative speculation from a firm foundation of scientific fact is invaluable and I rather think that Dr Lea's speculations and suggestions may well lead to the opening up of new horizons not only in radiation biology but also in genetical theory and practice and the study of bacteria and the viruses. His book is certainly one to be read and 'well thought on' by all workers in these fields.

WILLIAM B. BRIERLEY

Biology of Tissue Cells. By ALBERT FISCHER. Pp. 348. Cambridge University Press. 1946. 31*s.* 6*d.*

When Alexis Carrel reported in Paris in 1910 that he had been able to grow tissue cells in culture, his claims were received with incredulity. Gradually as acceptance came there followed a period when expectations as to what the study of cells in culture might reveal ran high—too high, for in course of time a reaction has set in, and what was regarded by its votaries as a new intellectual discipline has come to be regarded by the disillusioned as a mere technique of rather limited application.

Dr Albert Fischer, in his volume of essays on the biology of tissue cells, restores the balance. His book is an apologia for the science of tissue culture as a study that has illuminated many aspects of biology; as a source of new tools for the study of old problems. Recently it has even been used as a tool for the study of developmental physiology, for the study of form by experimental means. For the tissue culture has a characteristic radial form; and if a sector is removed, extra active growth in the injured zone gradually restores that form. The normal organism is a closed system; tissue cells in culture form an 'open system', the free margins of which are in a constant state of growth and movement in a fruitless effort to restore continuity. The study of such cells has naturally thrown much light on the processes of wound healing and regeneration; it has demonstrated the limitations of the cell theory, for the single tissue cell cannot thrive and multiply—only cells in contact or united by their cytoplasmic bridges are able to flourish. The cell migrations that are so characteristic and were so surprising a feature of tissue cultures are now known to figure largely in normal ontogeny. Tissue cells in culture provide perhaps the most promising material for the study of age and death.

These are a few of the topics discussed in full by Dr Fischer, with copious references. Among them are many observations made by the author in the course of years as Director of the Biological Institute in Copenhagen, hitherto unpublished. The whole is presented in a most readable and stimulating form. The book has been translated from the Danish and produced in Copenhagen, so that naturally there are a number of slips in our difficult language. But on the whole it successfully avoids the desiccated quality of an English translation.

V. B. WIGGLESWORTH

Gall Midges of Economic Importance. By H. F. BARNES, Agricultural and Horticultural Handbooks. London: Crosby Lockwood and Son, Ltd. 1946. Vol. I. Root and Vegetable Crops. Pp. 104. 12*s.* 6*d.* Vol. II. Fodder Crops. Pp. 160. 15*s.* 0*d.*

Dr Barnes has long been recognized as one of the world's leading authorities on gall midges and for some years economic entomologists everywhere have hoped that he would provide them with a monograph on this group of insects. Vols. 1 and 2 of a series of eight projected volumes on *Gall Midges of Economic Importance* by Dr Barnes will therefore receive a warm welcome.

It is a new and unique experience to read two volumes of a monograph or reference work on a group of insects and yet not to find a single detailed morphological description of one species or even genus: although not written for the systematist this is one of the most challenging aspects of Dr Barnes's work. Gall midges are amongst the most highly specialized of insects and it is the exception to find one species attacking more than one genus of plants. At first sight 'Diagnostic Characters' such as 'Folded leaflets of Lucerne' or 'Folded leaflets of Sanfoin' (vol. 2, pp. 51 and 52 respectively) would appear to be absurdly inadequate and yet they are, without question, all that is necessary to identify some species of gall midges. Conversely, so closely are some of these midges allied morphologically that it is extremely difficult and occasionally wellnigh impossible to identify with certainty a species from the imagines alone. The descriptions throughout are in fact an example of what has been termed 'the New Systematics' and it is very gratifying to have the first two parts of a monograph on these lines on an important group of economic insects from the pen of a British authority. For these very reasons, however, it is rather strange to find in this work plates such as 7 and 8 (vol. 1) and the Frontispiece of vol. 2, which are photographs of adult midges, and only a very modest sprinkling of plates depicting typical gall midge damage. The latter are extremely good and more of these would have enhanced the value of both volumes.

Some idea of the background of the work involved in the study of gall midges may be obtained when the author in the Preface states that he has been able to handle 3000 midges a day for eight weeks or so. The marshalling of all these data has been most painstaking and exact; the references have been most carefully and fully compiled and one striking feature is the very full acknowledgement that has been made throughout to all workers on midges and even to all entomologists and others who have submitted material to the author. The index too is very full and in three parts, a midge index, plant index, and general index. A very interesting and most useful feature is the notes provided on the economic importance of some of the lesser known host plants.

A few minor errors noted are: vol. 1: p. 27, 'larvae' for 'larva'; p. 61, 'Pl. 8, Figs. 1–2' for 'Pl. 8, Figs. 2–3'; and p. 89 'Rakmaninov' for 'Rakhmaninov'. Vol. 2: p. 69, 'Bew's' for 'Bews'; p. 77, '*Agrostic*' for '*Agrostis*'; and the use of 'Hymenopterous' with and without the capital. It seems a pity too to use the common name 'Red Clover Gall Gnat' (p. 27) when 'Midge', which has otherwise been used, is a much better term.

When completed the work will be of inestimable value to all economic entomologists and crop husbandry specialists. Many entomological specialists will probably bring pressure to bear on the author to prepare a companion series of volumes with morphological descriptions and keys. In the meantime all will look forward to the publication of the succeeding volumes—those promised are: vol. 3, *Gall Midges of Fruit*; vol. 4, *Gall Midges of Ornamental Plants and Shrubs*, and subsequent volumes on gall midges of trees, cereals, beverage plants and herbs, zoophagous and fungivorous midges and gall midges of weeds.

I. THOMAS

DDT The Synthetic Insecticide. By T. F. West, M.Sc., Ph.D. (Lond.), F.R.I.C. and G. A. Campbell, M.Sc. (Leeds), F.R.I.C. Pp. xii+301, with 13 plates. London: Chapman and Hall Ltd. 1946. 21*s.* 0*d.*

The potential usefulness of D.D.T. under war conditions mobilized intense scientific research on its chemical, physical and biological properties which, though applied primarily to its military use against the insect vectors of malaria and typhus, had a direct bearing on the possibility of employing the insecticide in other fields. Hence when supplies were released for non-military purposes, progress was astoundingly rapid and within two years a rich but scattered literature has accumulated. Dr West and Mr Campbell have reviewed this literature up to the autumn of 1945 and its extent is indicated by their author index of nearly 300 names.

The book opens with a brief 'prologue' relating the recruitment of the Swiss discovery of the insecticidal properties of D.D.T. to the allied war effort and it closes with an appraisal of its potentialities in peace, a stimulating challenge to the synthetic chemist and the biologist. Part I (pp. 12–109) is a survey of the chemical and physical properties of D.D.T. in relation to the conditions to be observed in its practical use and of methods whereby the chemical can be compounded to forms suitable for spray, dust or mist application or be incorporated into paints, textiles and paper. An important chapter reviews evidence of the action of D.D.T. on warm-blooded animals. Part II (pp. 110–286) reviews published results of trials of the insecticide against lice, mosquitoes and other pests noxious to man and animals. The account of its use against plant pests runs to nearly 100 pages.

The authors have accomplished their task with skill and accuracy. They have, when possible, given the results of comparative tests of the performance of D.D.T. with that of older insecticides and have so dispelled the fear, aroused by their choice of title and by the absence from their subject index of mention of other insecticides, of discrimination in favour of D.D.T. There is a remarkable freedom from misprints though the source of the initials D.D.T. is wrongly given on the inside cover of the wrapper as diphenyl-dichloro-trichlor-ethane. The flea beetle referred to in the footnote of p. 241 should presumably be *P. cruciferae* though the misprint may have appeared in the original source quoted. Indeed there are several signs that the authors are treading less sure-footedly in entomology than in chemistry. Thus, on p. 243, the sentence 'The insect *Leptinotarsa decemlineata* Say (especially in the larvae stage) appears...' suggests that the authors are unaware that they are discussing the notorious Colorado Beetle. A similar literal but uncritical quotation appears on p. 280 where in Table 20 the treatments applied are given in terms such as '3 lb. Lead in petal fall, 1 lb. D.D.T. and 3 oz. sodium lauryl sulphate in 6 other sprays' which are incomprehensible to a reader unfamiliar with the spray programme normally used in Maryland against Codling Moth.

The book will serve a most useful purpose in correcting popular misconceptions on the properties of this material which suffered more than it gained from the exuberant publicity its discovery received. But it will be a greater boon to the biologist who must know the proved capabilities and applications of D.D.T. To the latter, however, there is the danger that the book will quickly become out of date. The true magnitude of the difficulties which the authors have faced and overcome in this first edition will become apparent later when a critical examination of the merits and defects of new protective insecticides, of which D.D.T. is a first and highly effective representative, will be required. HUBERT MARTIN

Danish Review of Game Biology. Vol. 1, part 1, pp. 1–111. Copenhagen. 1945.

The first part of this new Danish scientific journal consists of a long paper by Holger Madsen entitled 'The species of *Capillaria* (Nematodes, Trichinelloidea) parasitic in the digestive tract of Danish gallinaceous and anatine game birds, with a revised list of species of *Capillaria* in birds'. This is a work of considerable taxonomic importance in a specialized and difficult

branch of helminthology, as species of the genus *Capillaria* present but few well-marked differential characters. Madsen lays stress, for diagnostic purposes, on the following anatomical features: for males, the size and shape of the spicule, the appearance of the spicule sheath and the shape of the tail end; for females, the presence or absence of a vulval appendage, the shape of the latter and the shape of the eggs. He also considers the location of the parasite in the alimentary tract as of systematic importance. The paper is divided into two main sections. In the first, species from the lower gut of gallinaceous game birds with related species from the fowl are dealt with. Here are given descriptions of the following: *Capillaria longicollis* (Mehlis, 1831), *C. dujardini* Travassos, 1915, *C. obsignata* nom.nov., *C. tiaras* n.sp., *C. cadovulvata* n.sp., and *C. collaris* (v. Linst. 1873). In the second part species from the lower gut of ducks, coot and goose are dealt with including the following: *C. anatis* (Schranck, 1790), *C. anseris* nom.nov., *C. mergi* n.sp., *C. nyrocinarum* n.sp., and *C. spinulosa* (v. Linst. 1890). Each species is fully illustrated with clear line drawings. Body measurements and proportions are expressed graphically and synonyms are fully listed. These two parts of the paper are followed, first, by an alphabetical list of *Capillaria* species from birds with authority and date, second, by a list of birds in which *Capillaria* species have been found and, third, by a table, occupying 8 pages, in which the most important features of bird *Capillarias* are set out. There is a useful summary and a full list of references. The whole publication is beautifully printed on good paper with a spaciousness of format and lay-out of the text which suggests that in Denmark they have not experienced the acute paper shortage which has been our lot in this country.

T. GOODEY